

ELECTROPHYSIOLOGICAL AND ANATOMICAL  
STUDIES IN THE RAT STRIATUM

by

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(i)

For  
Mum and Dad



(ii)

Statement in terms of Ph.D. Regulation 2.4.15 of the Postgraduate Regulations of the University of Edinburgh.

I declare that this thesis was totally composed by myself, and that all experimental work described herein was performed by myself with the following exceptions:

1. The PhAL injections and immunohistochemical localisation of CaBP in Chapter 2 were performed by Dr. C.R. Gerfen.
2. The transplantation of fetal striatal tissue in Chapter 4 was performed by Dr. S.B. Dunnett.
3. The effects of potassium concentration on afterhyperpolarisations in Chapter 5 were investigated by Dr. M. Garcia-Munoz.

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ABBREVIATIONS

AChe	acetylcholinesterase
AD	anterodorsal thalamic nucleus
AHP	afterhyperpolarisation
AM	anteromedial thalamic nucleus
APP	avian pancreatic polypeptide
ASP	aspartate
CaBP	calcium binding protein
ChAT	choline acetyl transferase
CL	centrolateral thalamic nucleus
CM	centromedian thalamic nucleus
CO <sub>2</sub>	carbon dioxide
DA	dopamine
DAB	3,3'-diaminobenzidine
EP	entopeduncular nucleus
EPSPs	excitatory postsynaptic potentials
FrPaM	frontoparietal cortex, motor area.
FrPass	frontoparietal cortex, somatosensory area
GABA	$\gamma$ -aminobutyric acid
GAD	glutamic acid decarboxylase
GDEE	glutamic acid diethyl ester
GLU	glutamate
GP	globus pallidus
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HRP	horseradish peroxidase
5-HT	5-hydroxytryptamine
IA	ibotenic acid
IPSPs	inhibitory postsynaptic potentials
KA	kainic acid
l	lateral
M	matrix
m	medial
MD	mediodorsal nucleus of thalamus
mfb	medial forebrain bundle
NA	noradrenaline
NMDA	n-methyl-d-aspartate

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O <sub>2</sub>	Oxygen
6-OHDA	6-hydroxydopamine
P	patches
PBS	phosphate buffered saline
PC	paracentral thalamic nucleus
PhAL	phaseolus vulgaris-leucoagglutinin
p1	paralamellar
PT	paratenial thalamic nucleus
QA	quisqualate
r	rostral
SN	substantia nigra
SNC	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SP	substance P
SS	somatostatin
TH	tyrosine hydroxylase
VIP	vasoactive intestinal polypeptide
VL	ventrolateral thalamic nucleus
VM	ventromedial thalamic nucleus
VTA	ventral tegmental area
WGA-HRP	wheatgerm agglutinin-horseradish peroxidase

ABSTRACT OF THESIS

This thesis examines anatomical and electrophysiological properties of the input to neostriatum from frontal cortex in the rat. This input is known to occur in a discontinuous fashion and the anatomical results define the differential input to striatal compartments from different groups of cortical neurones.

Extracellular recording experiments 'in vivo' cannot be used to examine the pattern of input in rat neostriatum because of the presence of corticofugal fibre bundles which pass through the nucleus. An 'in vitro' preparation was therefore developed in which slices were cut parallel to the corticofugal fibres thus allowing the distribution of extracellular activity from single neurones excited by cortical stimulation to be examined. A 'patchy' distribution of responsive neurones was seen as the electrode was moved along the grey matter between corticofugal fibre bundles. Some evidence of cortico-striatal input to embryonic neurones transplanted into damaged striatum was available from anatomical studies. Consequently a series of experiments designed to show electrophysiological responses in grafted neurones after cortical stimulation was performed. Responses were obtained which were similar to those seen in slices from intact animals, although the later phases of the response were certainly not identical.

Finally, the passive and active membrane properties recorded intracellularly from neurones in the slices are described. Responses to short depolarising intracellular pulses and to cortical input were both reduced by iontophoretic application of dopamine, while the number of action potentials following longer intracellular pulses was increased. This later "excitatory" effect seems to be a consequence of decreased accommodation which in turn results from an action of dopamine on the afterhyperpolarization which follows action potentials in striatal neurones. Although it is smaller and requires multiple action potentials to be easily measurable, the afterhyperpolarisation seems to be the result of an outward current carried by potassium ions and is perhaps similar to the calcium-activated potassium current seen in hippocampal neurones.

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## CHAPTER 1

### General Introduction

## INTRODUCTION

The striatum, which is the largest subcortical structure in the mammalian brain has been the subject of extensive study for well over a century. More recently, particular attention has been focused on it since biochemical and pharmacological investigations have highlighted its involvement in disorders of movement, particularly Parkinson's Disease (Hornykiewicz, 1963; Ehringer and Hornykiewicz, 1960) and Huntington's chorea (Mason and Fibiger, 1978; 1979; Bird, 1979).

The search for an understanding of the normal processes underlying these disease states is an important one, which has been hampered to a certain extent by the anatomical complexity of the striatum. In the case of Parkinson's Disease, a variety of confusing data has also arisen over the involvement in striatal neuron activity of dopamine (DA), the loss of which causes severe impairment of motor function.

The experiments in this thesis have been designed to gain a better understanding of the anatomical and electrophysiological properties of the striatum with particular reference to the input from the cerebral cortex and the effects of DA on neuronal activity within the striatum. In addition the ability of host cortex to innervate transplanted striatal tissue will be examined.

The literature review which follows describes the development of the current knowledge about the intrinsic organisation of the striatum and its major inputs and outputs, which is necessary for the understanding and interpretation of the results to follow.

### Definition

The approved term for anterior areas of the basal ganglia is corpus striatum (Kopsch, 1957) which encompasses the caudate nucleus, putamen and globus pallidus. In primates the caudate and putamen are distinct nuclei while in most other mammals, they are less clearly separated. The cellular homogeneity of these two nuclei has led them to be classified as one, referred to as the striatum. Thus, the term striatum will be used in this context throughout this thesis.

### LITERATURE REVIEW: THE INTERNAL ORGANISATION OF THE STRIATUM

This review is divided into two main parts:

1. Neuron types
2. Compartmental organisation of the striatum.

#### 1. Neuron types

Neurons in the striatum have been described using Golgi impregnation methods in human (Cajal, 1911; Graveland et al., 1985), monkey (Fox et al., 1971/1972a,b; Pasik et al., 1976, 1979; DiFiglia et al., 1976; Rafols and Fox, 1979), dog (Tanaka, 1980), cat (Kemp and Powell, 1971; Adinolfi and Pappas, 1968) and rat brain (Mensah and Deadwyler, 1974; Lu and Brown, 1977; Danner and Pfister, 1979; Dimova et al., 1980; Chang et al., 1982). Classification of these neurons has been made according to the size and shape of their perikarya, the number and structure of dendrites and axon length. Taking into account the wide variety of species used and the interpretational differences of the various studies, clear similarities between these reports exist which suggests that direct

TABLE 1 : CORRELATION OF STRIATAL NEURON TYPES (using classification of Graybiel and Ragsdale , 1983)

REFERENCE	SPECIES	SPINY I (medium ,densely spiny)	SPINY II (medium , long axon)	ASPINY I (medium , varicose dendrites)	ASPINY III (medium , smooth dendrites)	SPINY II (large spiny)	ASPINY II (large aspiny , short axon)	STRIATONIGRAL TYPE II (Bolam et al , 1981b)	NEUROGLIFORM
Cajal (1911)	human , mouse ,rat rabbit ,dog	long axon (Figs. 325B ,327A)		short axon (Figs. 325A ,326C)	short axon (Fig. 325D)	long axon (Fig. 325C)			neurogliform (Fig. 325E)
Leonovich (1954)	human , dog monkey , hedgehog , rabbit , mouse	rich dendrites , long axon		"spiral" , short axon	"stellate" short axon	poor dendrites, long axon	giant , short axon		spidery
Kemp and Powell (1971)	cat	medium spiny , short axon	medium , long axon	medium , varicose dendrites , short axon	medium , smooth dendrites short axon	"giant cell"?	large , long axon		oligodendrocyte
Fox et al (1971)	monkey	spiny , short axon		spidery , aspiny	aspiny with few dendritic spines		large aspiny , long axon		oligodendrocyte
Mensah and Deadwyler (1974)	rat	medium stellate		medium , varicose dendrites	medium , smooth		large		
Lu and Brown (1977)	rat	medium , long axon/ medium spidery	medium spindle	medium , varicose dendrites	medium , long slender dendrites		large		small
Danner and Pflster (1979)	rat	type 1		type 5? type 4?	type 2 and 5?		type 3		
Tanaka (1980)	dog	spiny I	medium spiny II	aspiny I	aspiny III/ medium spiny II?	large spiny II?	aspiny II		
Dimova et al (1980)	rat	type 1	type 2?	type 3 , type 4?	type 4? type 2?	type 5	type 5	type 5	neurogliform
Chang et al (1982)	rat	medium type I	medium type II	type IV? type V ?	medium type III	type II large	type I large		small
Bishop et al (1982)	rat	MS 1	MS 2		MA		LA?		
Graveland et al (1985)	human	spiny I	spiny II?	aspiny I		spiny II?	aspiny II		

comparisons of the results of physiological experiments in different species can be made.

The bases on which these classifications have been made have been consolidated by the use of ultrastructural studies which can reveal intracellular details. In addition, more recent work using intracellular markers applied locally or transported to cell bodies by retrograde transport has determined whether or not given neuron types can be considered as projection neurons or interneurons. On the basis of such work there are at present at least eight morphologically distinct types of striatal neuron (Chang et al., 1982; Pasik et al., 1979), some of which are detailed in Table 1 and Figures 1, 2 and 3.

These categories of neuron are discussed below using the classification system of Graybiel and Ragsdale (1983) which is a slightly modified version of that presented by Pasik et al (1979). Their inputs and outputs are summarised diagrammatically in Figure 4 (page 31).

### 1.1 Medium spiny type I

All studies employing Golgi impregnation techniques have described a medium-sized neuron with dendrites heavily covered in spines as being the most frequently impregnated class of neurons (Figure 1a). Most studies have claimed that approximately 90-96% of striatal neurons are of this type (DiFiglia et al., 1976; Dimova et al., 1980; Kemp and Powell, 1971; Chang et al., 1982). These estimates, however, should be treated with caution as Golgi impregnation is notoriously discriminatory and so it is possible that this class of neuron is preferentially labelled in studies of this type.



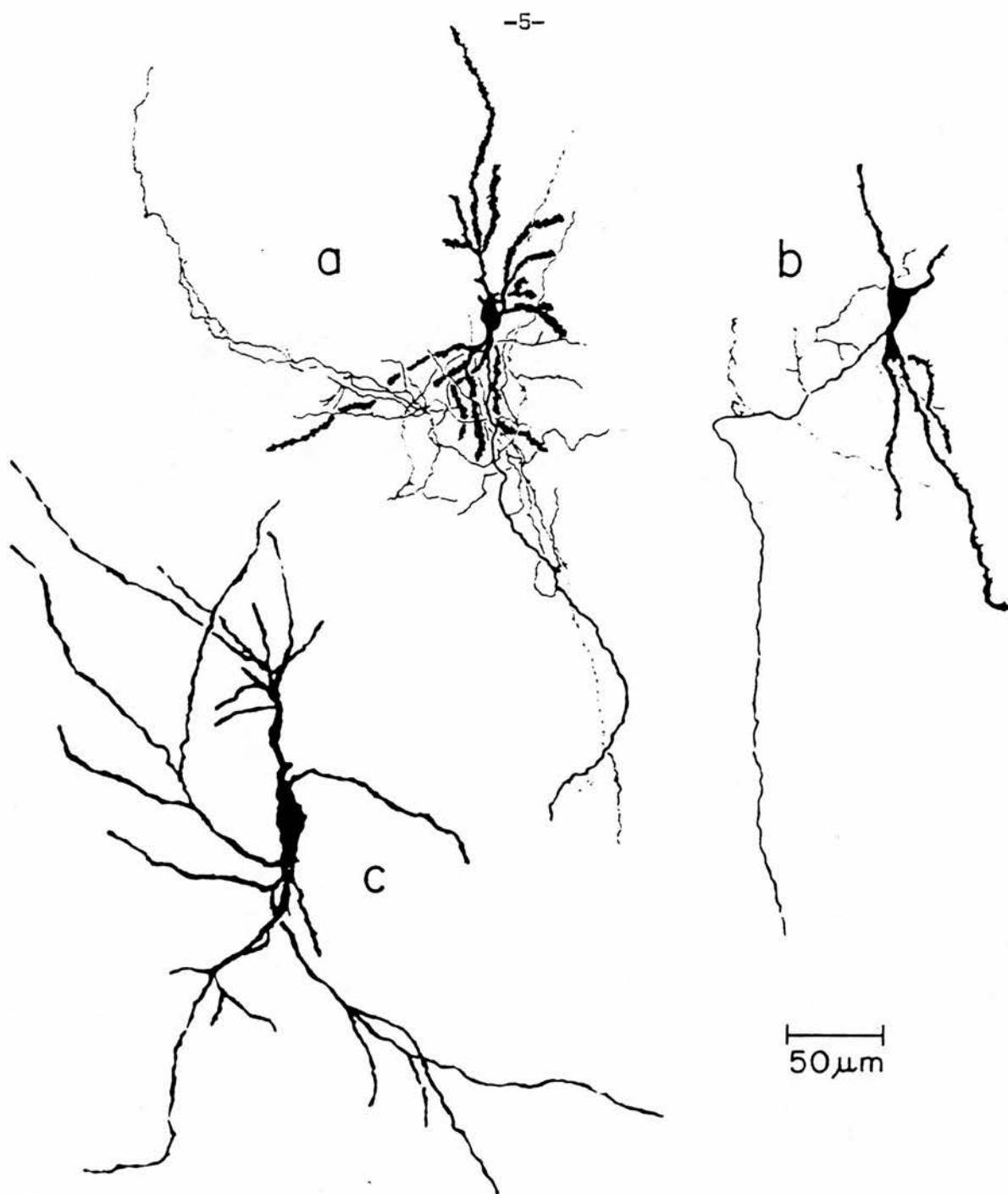


FIGURE 1: Spiny neurons

Camera lucida drawings of Golgi-Kopsch impregnated striatal neurons from monkey striatum.

- a. Spiny I neuron with densely spiny dendrites and a long axon with abundant collateral branches.
- b. Spiny II neuron with sparsely spiny dendrites and a long axon with some collaterals.
- c. Large version of Spiny II neuron with spines on the somatic and dendritic surfaces. The axon of this neuron has not been impregnated.

Redrawn from Pasik et al (1979).

(a) Morphology

The terms "medium spiny" or "medium-sized densely spiny" used to describe these neurons refer to their appearance in Golgi-impregnated material. In general the perikarya are oval or polygonal in shape and 10-18 $\mu$ m in diameter, with several dendrites (200-250 $\mu$ m long) emerging from them. The primary dendrites and initial parts of most secondary dendrites are spine-free, but more distal dendrites become densely laden with spines. Ultrastructural studies (Dimova et al., 1980; Pasik et al., 1976; Kemp and Powell, 1971) have demonstrated the presence of an ovoid nucleus which has no invaginations of the nuclear envelope or intranuclear inclusions, an important factor in the differentiation between this and other medium-sized neurons.

(b) Outputs

Early reports considered the medium spiny neuron to have a short axon which terminated either within or close to the limits of the dendritic arbor (300-400 $\mu$ m in diameter). These neurons were thus considered to be interneurons, which led to the view that the striatum was an integrative structure with less than 5% of its neurons as outputs (Vogt and Vogt, 1920; Fox et al., 1971/1972a,b; Kemp and Powell, 1971).

This view was initially challenged by Leontovich (1954) who carried out an extensive Golgi study in various species and found that the vast majority of medium-sized neurons possessed long efferent axons. This finding was confirmed by Grofova (1975) who observed numerous striatal neurons filled with Horseradish Peroxidase (HRP) from a nigral injection in monkeys. A large

proportion of these neurons were identified as medium spiny.

Subsequent retrograde transport experiments in the rat and monkey have confirmed this finding and have shown that approximately 50-70% of striatal neurons are efferent (Bunney and Aghajanian, 1976; Bak et al., 1978; Bolam et al., 1981a; Szabo, 1979) although it has to be emphasised (and see later) that not all striatonigral neurons are of this type.

Further confirmation of their role as striatonigral projection neurons comes from elegant studies carried out by Somogyi and Smith (1979) and Somogyi et al (1981a) using a combination of Golgi impregnation and gold-toning with the retrograde transport of HRP from the substantia nigra (SN). Similarly, Chang et al (1981) traced the axons of medium-sized spiny neurons identified by intracellular injection of HRP out of the striatum and demonstrated their arborisation within the globus pallidus (GP).

Another approach used to study distant synaptic connections of striatal efferent neurons is through the examination of degenerating synaptic boutons in contact with identified neurons in target areas after lesioning the striatum. By this method, striatal projection neurons have been shown to make symmetrical synaptic contact with nigrostriatal neurons (Somogyi et al., 1981b), tyrosine hydroxylase (TH)-immunoreactive dendrites in SN (Wassef et al., 1981), nigrothalamic neurons (Somogyi et al., 1979) and pallidonigral neurons identified by retrograde transport of HRP (Totterdell et al., 1984).

Until quite recently it was assumed that many striatopallidal axons were collateral branches of striatonigral fibres (Fox and Rafols, 1975; Yoshida et al., 1971, 1974) and also that striatal

axons in the two pallidal segments (GP and entopeduncular nucleus (EP) in rats) were collaterals of one another. However, several double labelling experiments have shown that these inputs arise from two separate neuronal populations in the squirrel monkey (Parent et al., 1984; Smith and Parent, 1986a) where axons terminating in GP were found to arise from putaminal neurons, while those projecting to SN arose mainly from caudate neurons. Similarly, Feger and Crossman (1984) found that only a small number of striatal neurons could be doubly labelled by deposits of two different fluorescent dyes in GP and SN, a finding which was confirmed in the cat (Beckstead and Cruz, 1986). A recent study by Gerfen (1984) has also demonstrated that neurons in the zona reticulata and zona compacta of the SN receive inputs from different populations of striatal neurons (see later).

(c) Axon collaterals

A further characteristic of these neurons is that they give off local axon collaterals within the striatum which are distributed in the same area as their dendritic arbor (Grofova, 1975; Wilson and Groves, 1980; Somogyi et al., 1981a; Bishop et al., 1982). These axon collaterals have been shown to make synaptic contact with other medium spiny neurons and other classes of neuron within the striatum (see sections 1(e), 1.6 and 1.7).

(d) Associated neurotransmitters and neurotransmitter candidates

Several experimental approaches have led to the conclusions that medium spiny neurons contain  $\gamma$ -amino-butyric acid (GABA) (Bradley et al., 1983; Morelli et al., 1983; Oertel and Mugnaini, 1983; Ribak et

al., 1979; Fisher et al., 1986b), substance P (SP) (Bolam et al., 1983b; Izzo and Bolam, 1986; Izzo et al., 1987), enkephalin (DiFiglia et al., 1982; Morelli et al., 1983; Pickel et al., 1980; Somogyi et al., 1982; Aronin et al., 1984; Izzo et al., 1987; Cuello et al., 1981; Sugimoto et al., 1987; Hokfelt et al., 1977), dynorphin (Vincent et al., 1982), taurine (Clark et al., 1984) and neurotensin (Goedert et al., 1983; Sugimoto et al., 1987). Of these identified substances GABA, SP and enkephalin have been proposed as transmitters in the striatonigral and striatopallidal pathways by means of electrophysiological (Precht and Yoshida, 1971; Crossman et al., 1973; Dray et al., 1976; Davies and Dray, 1976; Dray and Straughan, 1976; Walker et al., 1976) and anatomical and biochemical studies (Brownstein et al., 1977; Fonnum et al., 1974; Gale et al., 1977; Kim et al., 1971; Kanazawa et al., 1977; Jessel et al., 1978; Cuello and Paxinos, 1978; Kawai et al., 1987).

The fact that each of these putative transmitters can be identified within a single morphological neuron type raised the question of whether they might be in coexistence within these neurons. This seemed unlikely in view of the strong evidence to suggest that SP is associated more with the striatonigral projection (especially to SN compacta - Gerfen, 1984) than enkephalin, and vice versa in the case of the striatopallidal pathway (Brownstein et al., 1977; Correa et al., 1981; Cuello and Paxinos, 1978; Hong et al., 1977; Krause et al., 1984; Mroz et al., 1977; Staines et al., 1980). This evidence is also supported by the observations that dense immunocytochemical terminal staining for enkephalin but not SP is found in the GP, while the reverse is true for SN and EP (Beckstead and Kersey, 1985; Chesselet and Graybiel, 1983; Cuello

TABLE 2 : CHARACTERISED INPUTS TO MEDIUM-SIZED SPINY NEURONS (ADAPTED FROM BOLAM , 1984)

ORIGIN OF AFFERENT TERMINALS AND/OR TRANSMITTER	TYPE OF SYNAPSE	TYPE OF VESICLE	DISTRIBUTION	REFERENCES
Cortex (glutamate/aspartate)	asymmetrical	small , round	spine heads , distal dendritic shafts	Somogyi et al 1981a Bouyer et al 1984 Hattori et al 1978 Somogyi et al 1982
TH-positive (dopaminergic from SN compacta neurons)	symmetrical	large , pleomorphic	spine necks , distal dendritic shafts , perikarya , proximal smooth dendrites	Freund et al 1984 Bouyer et al 1984 Pickel et al 1981
ChAT-positive (cholinergic interneurons)	mainly symmetrical , some asymmetrical	large , pleomorphic	spine necks > distal dendritic shafts > perikarya and proximal smooth dendrites	Wainer et al 1983 Wainer et al 1984 Bolam and Izzo 1986 DiFiglia 1987
SP-positive (axon collaterals of medium spiny neurons)	symmetrical	large , pleomorphic	shafts > spines > perikarya (mainly proximal to perikarya)	Bolam et al 1983b Izzo and Bolam 1986 Bolam and Izzo 1988
GAD-positive (GABAergic medium spiny , or interneurons)	symmetrical (some asymmetrical on to shafts)	large , pleomorphic	spine necks , dendritic shafts , perikarya , proximal smooth dendrites	Wilson and Groves 1980 Ribak et al 1979 Bolam et al 1983
Enkephalin-positive (medium spiny neurons)	symmetrical (some asymmetrical on to shafts)	large , pleomorphic	spine necks , dendritic shafts , perikarya , proximal smooth dendrites	DiFiglia et al 1982 Somogyi et al 1982 Pickel et al 1980
Somatostatin-positive (interneurons)	symmetrical	large , pleomorphic	spine necks , distal dendritic shafts , perikarya , proximal smooth dendrites	Takagi et al 1983
5-HT-positive (from raphe)	asymmetrical	small , round	spines	Pasik et al 1981

N.B. Identification of the medium spiny neurons was by Golgi impregnation which was often coupled with retrograde labelling from SN.  
Several studies also used ultrastructural characterisation as a means of identification.

and Kanazawa, 1978; Graybiel, 1984; Haber and Nauta, 1983; Haber and Watson, 1983; Ljungdahl et al., 1978). Double labelling studies in the rat and cat have shown that while a small proportion of medium spiny neurons contained two transmitter substances, the vast majority were only labelled by one (Penny et al., 1986), although a report by Aronin et al (1984) demonstrated as many as 50% of enkephalin-positive neurons also contained glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA.

#### (e) Inputs

Several studies have described the organisation of synaptic inputs to the medium spiny neuron (Wilson and Groves, 1980; Somogyi et al., 1981a; Bishop et al., 1982) as forming either symmetrical or asymmetrical membrane specialisations. The former type have been shown to occur in all regions of postsynaptic neurons, that is, soma, initial segment, all dendrites, spines and shafts, whereas the latter specialisations are almost exclusively associated with the head region of dendritic spines.

Three sources for these inputs have been characterised: extrinsic, local axon collaterals, and interneurons (summarised in table 2).

##### (i) Extrinsic sources

While it has been shown that the striatum receives inputs from cortex, SN, thalamus, dorsal raphe, amygdala and GP (see Graybiel and Ragsdale (1983) for review), only the morphology of synapses from the cortex, SN and raphe on to identified medium spiny neurons have been described.



Corticostriatal afferents (which are thought to use glutamate (GLU) as a transmitter, see chapter 3) have been shown to form asymmetrical synapses with the expanded head region of dendritic spines and possibly dendritic shafts (Kemp and Powell, 1971; Hattori et al., 1978; Somogyi et al., 1982; Bouyer et al., 1984). Such synapses have been suggested to be the major type in the striatum, making up 80% of the total number (Pasik et al., 1976). However, Pickel et al. (1981) reported that 21% of synapses were dopaminergic, so considering that other types of synapse are present it is likely that one of these values is an overestimation.

Despite being the major type of afferent input, the input from any given cortical area to an individual medium spiny neuron has been shown to be relatively sparse (Somogyi et al., 1981a) although this type of synapse occurs very frequently on individual neurons. This can be explained by the fact that most cortical areas provide inputs that are distributed to broad areas of the striatum (Yeterian and van Hoesen, 1981; and see chapter 2).

Studies employing uptake of radiolabelled DA (Descarries et al., 1980), immunohistochemical localisation of TH (DA synthetic enzyme) (Pickel et al., 1981; Bouyer et al., 1984; Arluison et al., 1984; Freund et al., 1984) and immunohistological localisation of DA (Voorn et al., 1986) have shown that dopaminergic afferents from SN make mainly symmetrical synapses with dendritic shafts and spines although a few have been found in contact with perikarya and proximal dendrites. Freund et al (1984) calculated that 6% of these synapses were on perikarya, 33% on distal dendritic shafts and 59% on spines.



TH-immunoreactive fibres have also been shown to synapse on the necks of dendritic spines (Freund et al., 1984) which also receive asymmetrical synapses of the type thought to originate from cortex. This organisation was confirmed by Bouyer et al (1984) by the demonstration that degenerating corticostriatal afferents make synaptic contact with dendritic spines that also receive TH-immunoreactive synapses, although this was not observed by Hattori and Fibiger (1982) after 6-hydroxydopamine induced lesions of the striatonigral pathway.

Pasik et al (1981) have also demonstrated ultrastructurally that 5-hydroxytryptamine (5-HT) positive synapses from the dorsal raphe nucleus form asymmetrical synapses on the spines of identified medium spiny neurons.

#### (ii) Local axon collaterals

Wilson and Groves (1980) determined that of synapses formed by local axon collaterals with medium spiny neurons, 12% are on to smooth proximal dendrites or perikarya, 48% on distal interspinal dendritic shafts and 40% on spines. Those synapses on to spines were reported to be most often located on the neck region of spines which also received an asymmetrical input to their head portion of the spine, presumably from a cortical afferent axon.

As mentioned in section (d), medium spiny neurons have been shown to express a variety of transmitter substances and enzymes. Several such reports also describe presynaptic terminals within the striatum expressing each of these substances which are similar in morphology to those of medium spiny axon collaterals and make similar types of contacts. The predominant synaptic type has been

shown to be symmetrical although a small number of GAD (Ribak et al., 1979) and enkephalin (DiFiglia et al., 1982; Pickel et al., 1980) immunoreactive boutons have been described to make asymmetrical synaptic contacts with the base of dendritic spines.

A considerable amount of work has been reported on SP-immunoreactive synapses (Bolam et al., 1983b; Izzo and Bolam, 1986; Bolam and Izzo, 1988). They have been found to form symmetrical specialisations on distal dendritic shafts (72.5%), spines (15%) and perikarya (10.7%) of medium spiny neurons. This pattern of input appears to be markedly different from other inputs by virtue of the large proportion of contacted perikarya.

Local axon collaterals of medium spiny neurons have been suggested to provide recurrent inhibition amongst local medium spiny neurons (Park et al., 1980; Levine et al., 1986) which is mediated by GABA (Katayama et al., 1981; Lighthall et al., 1981). The synaptic relationship of local axon collateral synaptic contacts with medium spiny neurons is consistent with this. However, the demonstration that enkephalin and SP are contained within medium spiny neurons and their collaterals suggests they too can directly affect the output of the striatum perhaps indirectly by the release of GABA from output neurons.

### (iii) Striatal interneurons

The inputs arising from these neurons will be dealt with in the appropriate sections (1.3, 1.4 and 1.6).

## 1.2 Medium spiny type II

A second type of medium spiny neuron was observed in the studies of Kemp and Powell (1971) and Lu and Brown (1977) and was positively identified as a separate type by DiFiglia et al (1976) (Figure 1b). Subsequent studies have also identified a neuron type with similar characteristics (medium sized II of Dimova et al., 1980; MS2 of Bishop et al., 1982; and probably type II of Chang et al., 1982).

Neurons of this type occur much less frequently than those of the spiny I type and differ from them in several ways. For instance, they possess sparsely distributed spines on their primary as well as high order dendrites. The primary dendrites also branch closer to the perikarya than those of spiny I neurons while the dendritic arbor of each is less dense.

Due to the rarity of these neurons very little work has been carried out on their inputs, outputs and neurochemistry per se. However, from descriptions of neuronal types in certain studies, some characteristics can be inferred. For instance it seems likely that neurons of this type are striatal output neurons by virtue of their long axons (DiFiglia et al., 1976). Various studies have mentioned the fact that not all outputs were from spiny I neurons (Grofova, 1975; Bunney and Aghajanian, 1976; Somogyi and Smith, 1979) although they were all of medium size, suggesting that two subtypes were present. Morphological evidence in support of this argument comes from the Golgi study of DiFiglia et al (1976) which demonstrated the presence of two types of medium spiny output neurons, and more recently from Fisher et al (1986a) using single and double labelling with connectivity and Golgi markers to show that medium spiny neurons possessed a range of anatomical

specialisations; some had folded nuclear envelopes, somatic and/or proximal dendritic as well as distal dendritic spines. It seems likely therefore that at least some of these were spiny II neurons. A recent study by Dube et al (1988) has demonstrated that thalamic inputs are preferentially located on the shafts of neurons with similar characteristics. These synapses were shown to be asymmetrical.

Several studies have indicated that these neurons contain similar neurotransmitter substances to spiny I neurons. Fisher et al (1986b) demonstrated that some GABA neurons in the cat striatum possessed characteristics suggestive of spiny II neurons. Similarly other studies have demonstrated SP-immunoreactive perikarya which seem to fit this description (Bolam et al., 1983a; Izzo et al., 1987; Izzo and Bolam, 1988) although they suggest that the substance P may be contained within a type of interneuron.

Obviously the neurochemistry of these neurons requires further elucidation but the fact that they have been shown to be projection neurons makes it likely that their neurochemistry would be similar to that of spiny I neurons.

A role for spiny II neurons within the striatum has been tentatively suggested by Chang et al (1982) on the basis of their local axonal arbors. Collaterals were shown to extend further and were more diffuse than those of spiny I neurons although they formed similar contacts (i.e. on shafts and spine necks). This prompted these authors to postulate that spiny II neurons may play a role in the integration of information from disparate areas of the striatum. Pasik et al (1979) have also postulated that these neurons probably mediate an excitatory output to the SN using SP as their transmitter substance, although this remains to be confirmed.

### 1.3 Aspiny type I

It has been demonstrated in various studies that aspiny type I neurons are of medium size, the perikarya of which often have a polar appearance (Figure 2c). They have long, varicose and often curved, almost spine-free dendrites which branch infrequently. When branching does occur this is usually close to the perikarya. Golgi studies have shown that these neurons possess beaded axons which form extensive local arborisations; this observation has led to the view that these are interneurons. According to Kemp and Powell (1971) and DiFiglia et al (1976) neurons of this type possess deeply indented nuclear envelopes.

Somatostatin-like immunoreactivity has been identified in medium-sized neurons in the striatum of the rat (Bennett-Clarke et al, 1980; Finley et al, 1981; Johansson and Hokfelt, 1980), cat (Graybiel et al, 1981), guinea pig (Feldman et al., 1979) and monkey (Beal et al, 1987). Various studies have since suggested that these neurons are specifically of the medium aspiny I type (DiFiglia and Aronin, 1982; Takagi et al, 1983; DiFiglia and Aronin, 1984) as they have nuclear indentations and varicose dendrites. Furthermore, experiments combining retrograde labelling with immunohistochemistry suggest that somatostatin-immunoreactive neurons do not project out of the striatum (Chesselet and Graybiel, 1986).

Somatostatin-immunoreactivity has been suggested to coexist in these neurons with neuropeptide Y-immunoreactivity in monkey (Beal et al, 1987; Smith and Parent, 1986b), rat (Beal et al, 1986) and cat (Smith and Parent, 1986b) striatum as well as with avian pancreatic polypeptide-immunoreactivity (APP) in rat (Vincent et al, 1982) and cat (Chesselet and Graybiel 1986). It has also been

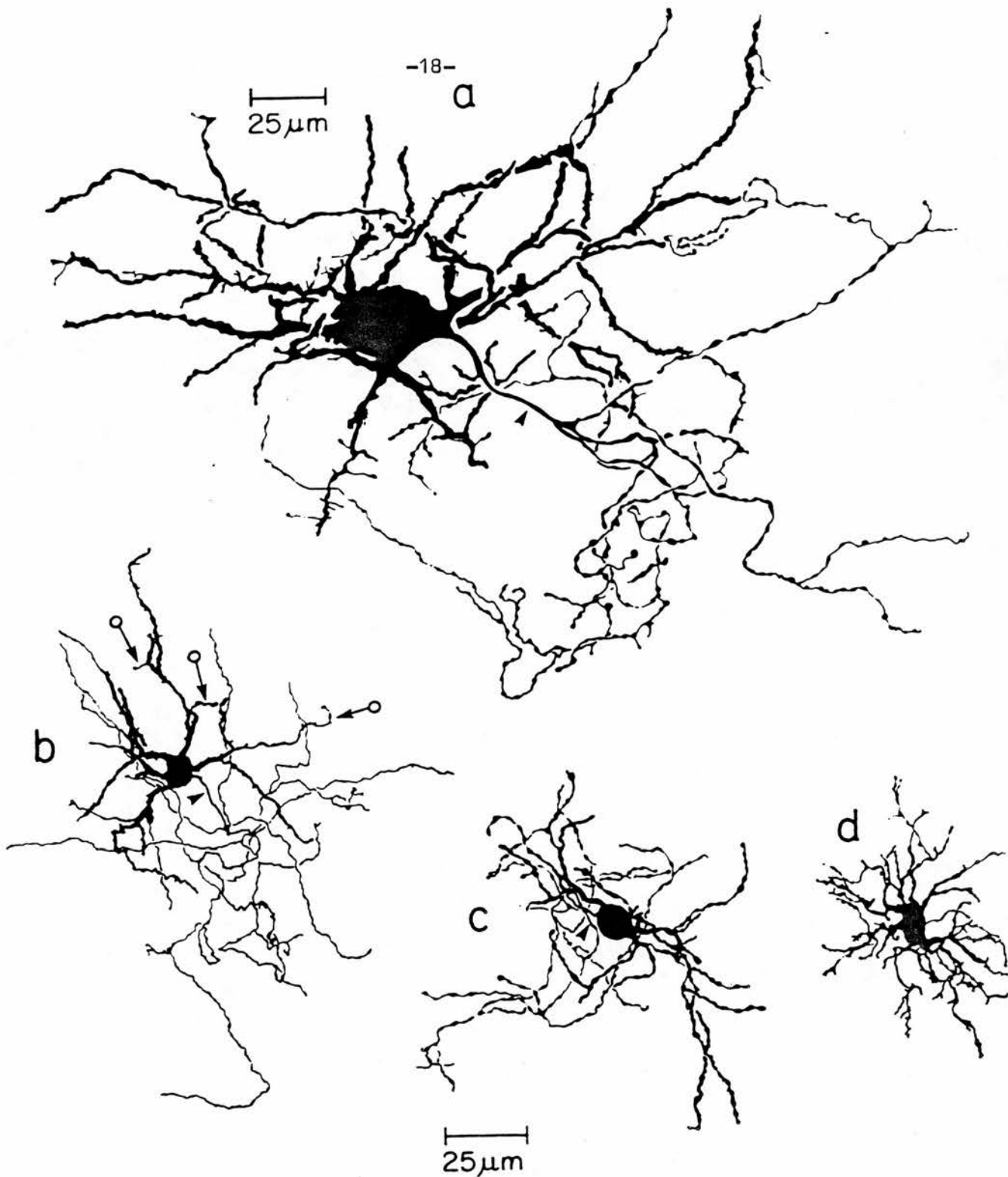


FIGURE 2: Aspiny neurons

Camera lucida drawings of Golgi-Kopsch impregnated striatal neurons from monkey striatum. The short axons of these neurons are indicated by the arrowheads.

- a. Aspiny II neuron. The "giant" striatal interneuron
- b. Aspiny III neuron with spine-like processes and dendritic appendages (ringed arrows).
- c. Aspiny I neuron. The "spidery" aspiny neuron of medium size with varicose dendrites.
- d. Neurogliform neuron.

Redrawn from Pasik et al (1979).

demonstrated however that those neurons immunoreactive for both somatostatin and APP also stain for NADPH-diaphorase (Vincent and Johansson, 1983; Sandell et al., 1986).

Afferent synapses on these neurons are relatively sparse and consist mainly of large boutons forming symmetrical synapses. Asymmetrical synapses are observed only rarely (DiFiglia and Aronin, 1982; Takagi et al, 1983). Proximal dendrites receive both types of synapse whereas more distal dendritic portions receive primarily asymmetrical synapses similar to those of corticostriatal inputs.

The outputs of aspiny type I neurons have been studied in Golgi-impregnated material (Takagi et al, 1984) and in sections incubated to reveal somatostatin-immunoreactivity (DiFiglia and Aronin, 1982, 1984; Takagi et al, 1983). Within the striatum somatostatin immunoreactive boutons possess large pleomorphic vesicles and form symmetrical synapses with dendritic shafts (73%) and spines (26%) and only very rarely with perikarya (1%) of spiny neurons amongst others (DiFiglia and Aronin, 1984). It has been reported however, that when axonal varicosities of somatostatin-immunoreactive axons were traced from their parent somata, they did not appear to form classical synaptic contacts (Takagi et al, 1983) but were in direct apposition (via membrane specialisations) to unlabelled perikarya or dendrites.

A role for these neurons is discussed in Part 2 of this review.



#### 1.4 Aspiny type III

The aspiny type III neuron of DiFiglia et al (1976) has been described as having a medium-sized perikarya approximately 12 $\mu$ m in diameter (Takagi et al, 1984) and giving rise to four primary dendrites which branch about 15-20 $\mu$ m from the soma (Figure 2b). These dendrites are long and more slender than those of the aspiny type I neuron and may only possess occasional spines on the more distal parts, rendering them essentially smooth. The axon originates from the perikarya and displays an elaborate and highly branched local collateral arborisation which extends some way beyond the dendritic field. In contrast to aspiny I neurons, these collaterals have been shown to branch frequently and also possess more frequent and larger varicosities.

Of the very few synapses which have been found to occur on the perikarya of neurons of this type (Takagi et al, 1984) both symmetrical and asymmetrical contacts were observed. Similarly dendrites also received both types of synapses although asymmetrical contacts were more frequent on distal dendrites. Axonal boutons from this neuronal type have been observed to make symmetrical synaptic contact with dendritic spines and shafts of postsynaptic elements (Takagi et al, 1984). It was also noticed that one of these postsynaptic dendrites originated from the same neuron as the axonal bouton thus representing an 'autapse'.

On the grounds of both dendritic characteristics and ultrastructure, medium aspiny type III neurons have been suggested to be similar to some of those neurons characterised as GABAergic by immunocytochemical studies using antibodies to GAD (Ribak et al, 1979, 1981; Panula et al, 1981; Bradley et al, 1983; Oertel and



Mugnaini, 1983; Bolam et al, 1985) and by virtue of their uptake of locally administered [ $^3\text{H}$ ] GABA (Bolam et al, 1983a) (although it has to be noted that in this case, no medium spiny neurons were labelled). Similarly, the inputs to these proposed GABAergic interneurons are very similar to those reported by Takagi et al (1984) for medium aspiny III neurons (Bolam et al, 1984a; DiFiglia et al, 1980).

Bolam et al (1985) have indicated that GABAergic interneurons make symmetrical synaptic contact with most other neuron types in the striatum including striatonigral neurons (see also Aronin et al, 1986). Indeed some of the boutons that form symmetrical contact with the perikarya and proximal dendrites of GAD-immunoreactive neurons are themselves immunoreactive for GAD (Ribak et al, 1979; Bolam et al, 1985) and probably arise from other GABA interneurons or GABA-containing medium spiny neurons.

A recent study also suggests that vasoactive intestinal polypeptide (VIP)-immunoreactive neurons are of this type (Theriault and Landis, 1987). They were shown to receive both symmetrical and asymmetrical synapses. Interestingly these also formed the occasional symmetrical contact with other perikarya which were immunoreactive for VIP. The possibility that VIP-immunoreactivity is in coexistence with GAD-immunoreactivity in these neurons remains to be explored.

### 1.5 Large spiny type II

These neurons are considered to be the large-neuron counterpart of the medium spiny II neurons and are distinguishable from them because of their size (Figure 1c). They have been demonstrated to

have elongated perikarya (30–50 $\mu$ m) and sparse spines on the primary and distal dendrites and appear similar to the single class of large neurons ("giant cells") recognised by Kemp and Powell (1971).

Little is known about these neurons due to the lack of agreement over the classification of large neuron types in the striatum. For instance, DiFiglia et al (1976) suggested that in the monkey, two distinct classes existed while Chang et al (1982), Rafols and Fox (1979) and Tanaka (1980) distinguished only one class in a variety of other species.

Pasik et al (1976) suggested that the large spiny neurons are projection neurons, although difficulty arose in attempts to sample large numbers of these neurons identified with respect to both their axonal projections and somatodendritic morphologies. This difficulty still exists and as a result few studies have been concerned with this type of neuron in particular. However, a type of large neuron has been found on occasions after injections of retrograde tracer into the medial pallidum (Graybiel et al, 1979; Mehler, 1981) but full cytological descriptions of the labelled neurons were not presented. Similarly, other studies have reported the retrograde labelling of large neurons after injection of Herpes simplex virus (Bak et al, 1978) HRP (Bolam et al, 1981b; Grofova, 1975; Szabo, 1979; Fisher et al, 1986a) into the SN, although Bolam et al (1981b) identified them as aspiny neurons (see section 1.6). A further striatal efferent system has been reported to originate from large neurons in the rat, namely an interhemispheric striato-striatal projection (Bak et al, 1981). The neurons involved in this projection were reported to be large spiny neurons with lobulated nuclei and perikaryal diameters of up to 50 $\mu$ m.

Several studies have indicated that, in the rat, neurons which are immunoreactive for choline acetyl transferase (ChAT) show similar characteristics to those of large spiny II neurons (Bolam et al, 1984b; Phelps et al, 1985), although the subcellular features of these cholinergic neurons have since been shown to be remarkably similar to aspiny type II neurons (DiFiglia, 1987 and see section 1.6). Pasik et al (1987) on the other hand recently observed neurons of this type which displayed GABA-immunoreactivity.

The possibility still exists that in some studies these large spiny neurons have been categorised as medium spiny II neurons. For instance, Graveland et al (1985) in their Golgi study on the human striatum identified a medium to large spiny neuron which they termed spiny II. It is possible therefore that a tighter control over the definition of medium and large-sized neurons is required. While such controversy exists, no definitive work has appeared in the literature which specifically focuses on the inputs and outputs of this type of neuron.

### 1.6 Aspiny type II

According to DiFiglia et al (1976) these are amongst the largest neurons within the striatum (diameter 25–30 $\mu$ m) and are equivalent in many respects to the medium sized aspiny type I neurons. Neurons of this type possess an oval perikaryon containing an invaginated nucleus and long (250 $\mu$ m), smooth, sparsely branching dendrites with abundant varicosities (Figure 2a). Originally they were considered to be output neurons (e.g. Kemp and Powell, 1971; Fox et al, 1971/1972) as they were thought to possess long axons. However, none of the retrograde transport experiments on striatal efferents

has found labelled neurons of this type and it is now accepted that their axons are short and arborise extensively within the striatum.

It has been suggested that aspiny II neurons are cholinergic in nature on the basis that they are stained by acetylcholinesterase (AChE) histochemistry (Sato et al, 1983; Bolam et al, 1984a; Parent et al, 1981). This has been confirmed by the immunohistochemical localisation of ChAT-immunoreactivity, which is a more specific marker for cholinergic neurons than AChE (Bolam et al, 1984b; Levey et al, 1983; Phelps et al, 1985; DiFiglia, 1987).

Various studies have reported that they are sparsely distributed in primate (Mesulam et al, 1984) and rodent (Kimura et al, 1980; Sofroniew et al, 1982; Levey et al, 1983) striatum. Quantification of the numbers of neurons immunoreactive for ChAT indicate that they account for 1.7% of the total neuronal population in the rat striatum (Phelps et al, 1985). Previous estimates of large neurons (the majority of which are presumed to represent aspiny type II neurons) in Nissl-stained sections of the monkey striatum have been in the range of 0.7-2.0% (see Pasik et al, 1979).

Several studies have demonstrated very high concentrations of acetylcholine in the striatum (Fonnum and Walaas, 1979). There is also agreement based on biochemical, pharmacological and morphological studies that the transmitter originates mostly from interneurons (McGeer et al, 1971, 1976; Katoaka et al, 1974; Woolf and Butcher, 1981) although it has been demonstrated that a small number of large AChE positive neurons project to the cortex (Parent et al, 1981) but it is unknown if these correspond specifically to aspiny type II neurons.

Studies on Golgi-impregnated large aspiny neurons (DiFiglia and Carey, 1986) and ChAT-positive neurons (DiFiglia 1987) have further confirmed the similarities between the two types of neuron. Synaptic inputs to both types have been found to consist of boutons forming both symmetrical and asymmetrical membrane specialisations on all portions of the neurons with the heaviest innervation occurring on distal dendrites (Phelps et al, 1985; Bolam et al, 1986) while the perikaryal input is sparse (Bolam et al, 1984a,b; Chang and Kitai, 1982; Takagi et al, 1984; Wilson et al, 1983; Bolam et al, 1986).

Symmetrical synapses have been shown to be most common on perikarya and proximal dendrites. A portion of these synapses have been demonstrated to contain SP-immunoreactivity and thus are likely to have arisen from the local axon collaterals of medium spiny neurons (Bolam et al, 1986). In contrast, asymmetrical synapses are most abundant on secondary and tertiary distal dendrites. The origin of these synapses has yet to be determined but the majority are most probably cortical.

Freund et al (1984) found little ultrastructural evidence for TH-immunoreactive nigrostriatal afferent input to cholinergic neurons. However, a recent study by Kubota et al (1987) demonstrated the presence of TH-immunoreactive synaptic contacts on the perikarya and proximal dendrites of these neurons.

Striatal cholinergic neurons have been shown to have extensive axon collaterals in striatum (Bolam et al, 1984b; Phelps et al, 1985). By analysis of the axons of Golgi impregnated neurons (Takagi et al, 1984) or HRP injected neurons (Wilson et al, 1983) local boutons have been reported to make symmetrical synaptic

contacts with dendritic spines or shafts. Wainer et al (1983, 1984), using monoclonal antibodies to ChAT, indicated that one target of cholinergic terminals is the medium spiny I neuron with the heaviest input to proximal regions. Bolam and Izzo (1986) and Izzo and Bolam (1988) confirmed this finding and also demonstrated that ChAT-immunoreactive boutons made symmetrical synapses with medium spiny striatonigral projection neurons, amongst others, with 45% of synapses being on shafts, 34% on spines and 20% on perikarya.

DiFiglia (1987) extended some of these findings. She reported that 53% of cholinergic synapses were on spines, while 37% were on shafts of primary and distal dendrites. A relatively small proportion of the boutons contacted axon initial segments (1%) and the perikarya of aspiny as well as medium spiny neurons (9%). It was also observed in this study that ChAT-immunoreactive boutons converged with unlabelled axons on to the same dendritic spines. Consequently it was suggested that intrinsic cholinergic axons may modulate extrinsic inputs on to striatal medium spiny neurons at postsynaptic sites close to the site of afferent input.

### 1.7 Large aspiny striatonigral type II neurons

With the observation that a least two putative transmitters - GABA and SP - were present in the striatonigral pathway (Brownstein et al, 1977; Gale et al, 1977; Kanazawa et al, 1977; Jessell et al, 1978; Staines et al, 1980), it seemed likely that at least two types of neurons might give rise to this projection. As already mentioned, a second type of medium spiny neuron with a long axon has been observed, but several lines of evidence have led to the suggestion that a third type of neuron may also be involved. Bak et

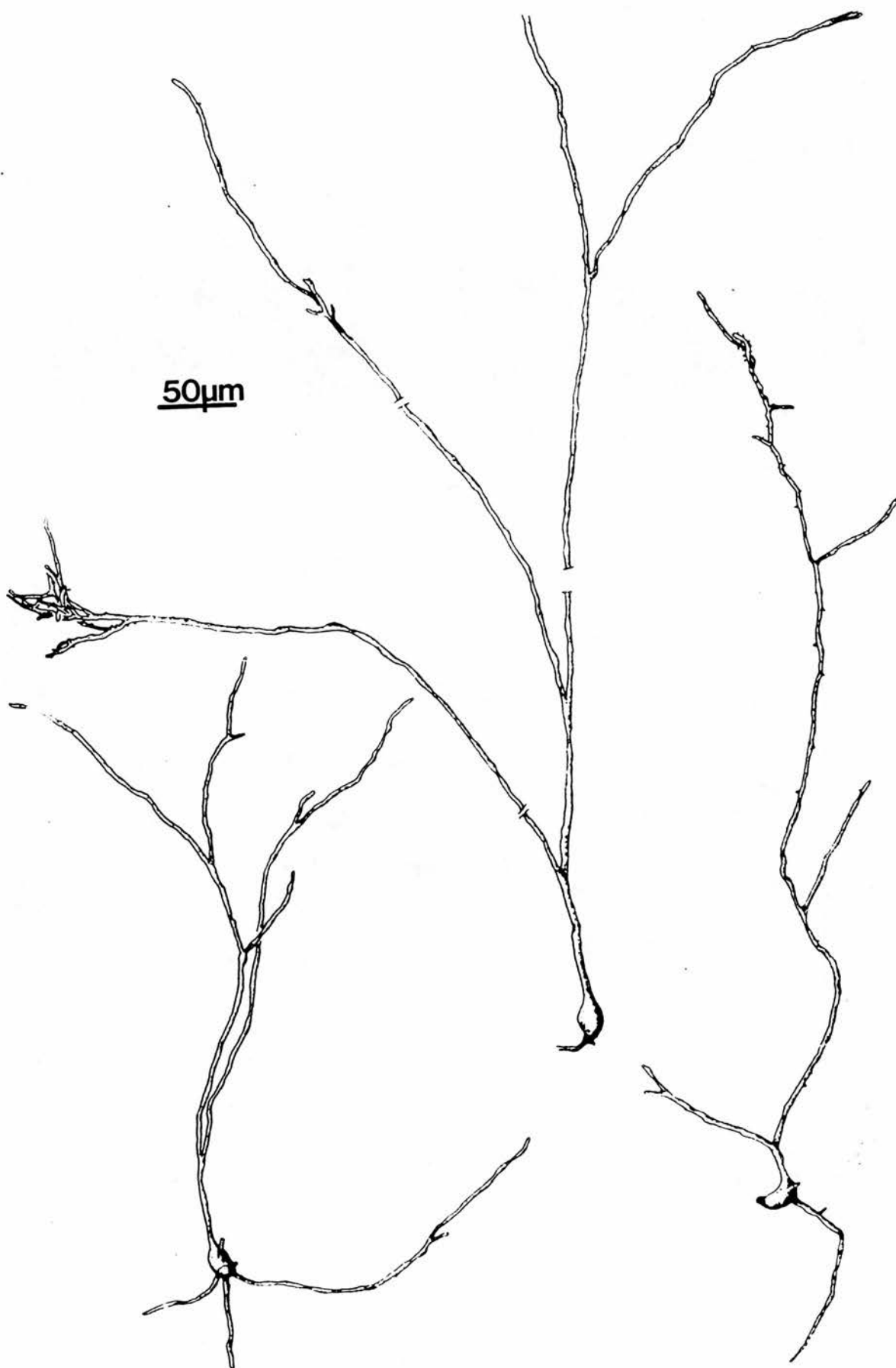


FIGURE 3: Striatonigral type II neurons.

Camera lucida drawings of three Golgi-stained neurons from rat striatum.

Adapted from Bolam et al (1981b).



al (1978) found a small population of large neurons in striatum after injection of Herpes simplex virus into SN, while similar studies in cat and monkey using HRP (Szabo, 1979; Grofova, 1975) demonstrated the occasional large striatal neuron retrogradely labelled with HRP.

Confirmation of this type of striatonigral projection neuron came from a study carried out by Bolam et al (1981b) in the rat, using retrograde HRP labelling (Figure 3). They found that not only were these neurons very rare, but they were only found in the ventral striatum.

Major differences occur between these and the other identified striatonigral neuron, the medium spiny neuron; they are larger (20-30 $\mu$ m in diameter), have indented nuclei and have a much greater density of synaptic boutons on their surfaces. Electron microscope analysis showed that they have very long (up to 700 $\mu$ m) essentially smooth dendrites (i.e. aspiny) which branch infrequently and lack varicosities. In many ways these neurons were similar to typical nigral or pallidal neurons. It could also be argued that they are larger medium spiny II neurons despite having more cytoplasm and heavier axosomatic input.

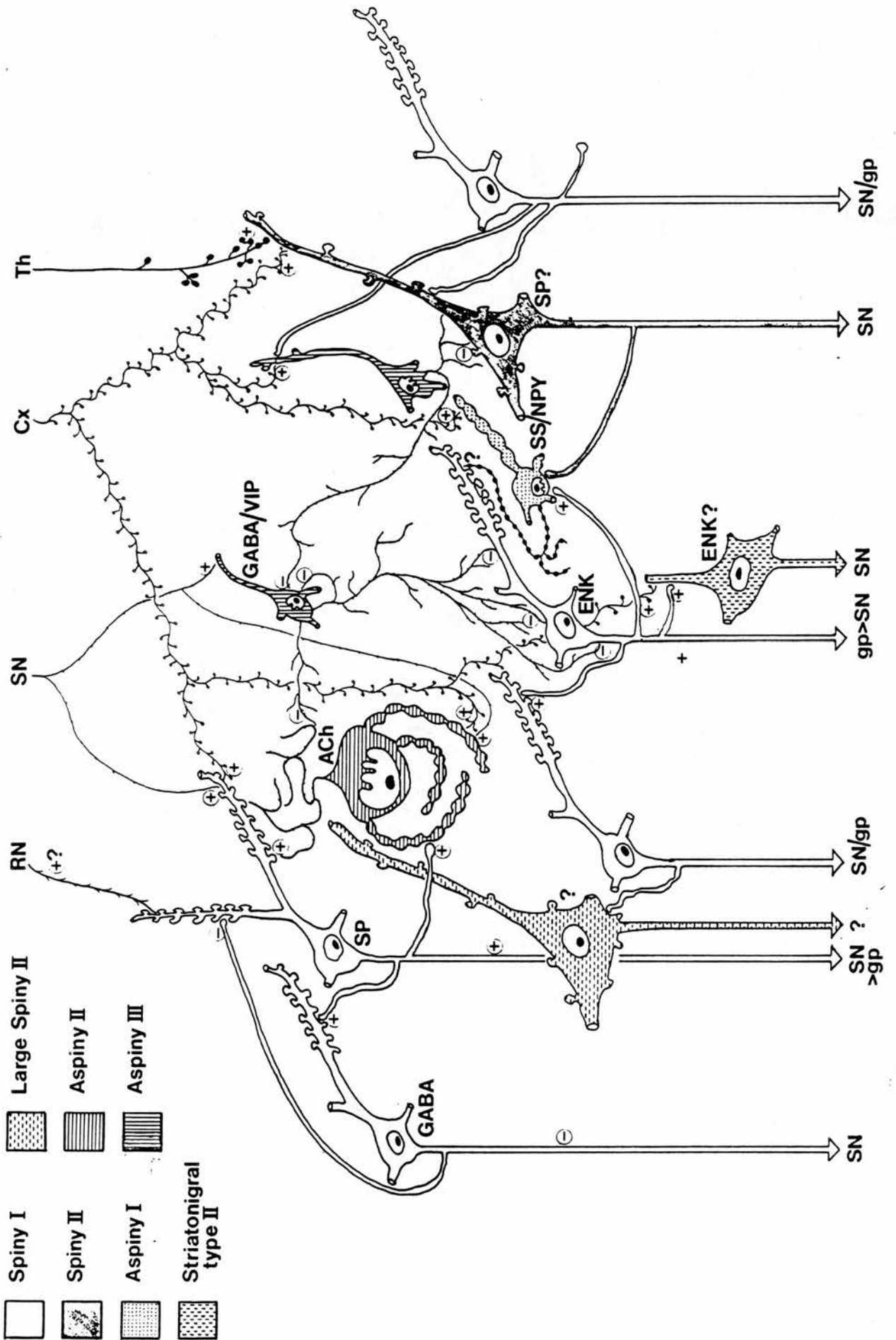
The synaptic input to these neurons consisted of numerous boutons of similar morphology which displayed symmetrical synapses on perikarya and proximal and distal dendrites. These synaptic boutons appeared very similar to boutons of local axon collaterals of striatonigral type I neurons (Bolam et al, 1980; Somogyi et al, 1981a,b). Subsequent studies have identified two chemically characterised inputs by using antisera to either enkephalin or GAD (Somogyi et al, 1982; Bolam et al, 1985) which are of the



FIGURE 4: Summary of neuronal circuits in the striatum. Shown in the diagram are known contacts of extrinsic afferents from cortex (Cx), thalamus (Th), substantia nigra (SN) and raphe nucleus (RN). Excitatory and inhibitory synapses are indicated by + and - respectively.

Putative transmitter substances are indicated close to the elements which contain them.

Full details are contained within the text.



symmetrical type. Asymmetrical contacts were also demonstrated although they were absent from perikarya and occurred rarely on proximal dendrites. As outlined in section 1.1(e) these are the major type of afferent bouton in the striatum, originating in mainly cortex.

Thus these neurons receive the same afferents as medium spiny type I neurons and appear to project to one of the same target areas. It remains to be determined whether they possess axon collaterals themselves, but it has been suggested that medium spiny neurons could exert a powerful feedback influence on these neurons via their own axon collaterals (Bolam et al, 1981b).

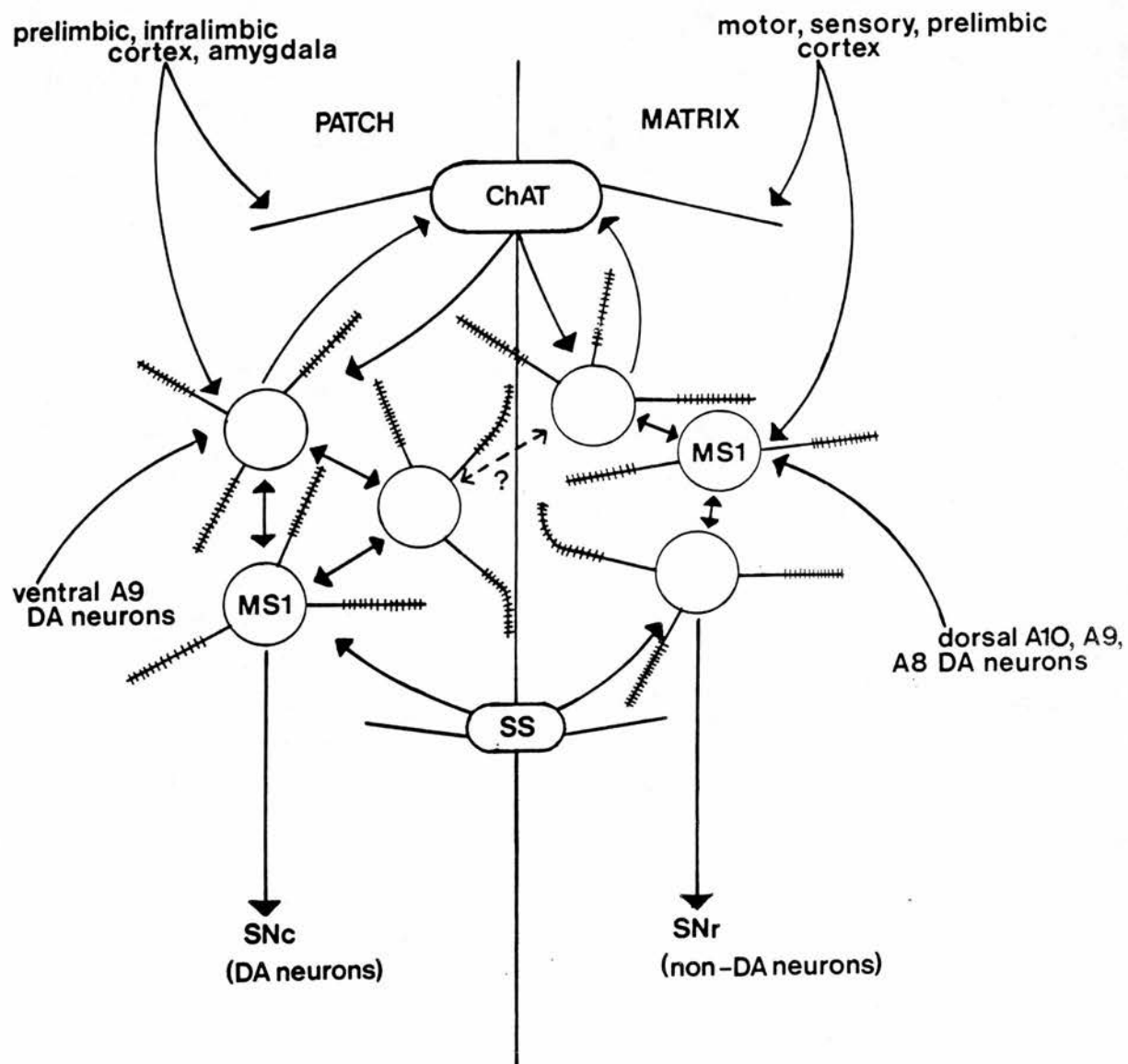
The nature of the transmitter(s) contained within these neurons also remains to be elucidated. Beckstead and Kersey (1985) reported that enkephalin-immunoreactive neurons were most numerous in the ventral part of the head of the striatum in the cat, and that in SN only a small number of striatal axons were immunoreactive for enkephalin. Thus it is possible that the striatonigral type II neurons may be responsible (in part) for the enkephalinergic projection to the SN, although at present no direct evidence for this exists.

### 1.8 Neurogliform cells

Small, dwarf or neurogliform cells (< 10 $\mu$ m diameter) were first described by Cajal (1911) and subsequent Golgi studies have reported their presence (Danner and Pfister, 1979; Kemp and Powell, 1971; Lu and Brown, 1977; Mensah and Deadwyler, 1974; Pasik et al, 1979). An example of this type of cell is shown in figure 2d.

FIGURE 5: Summary diagram of the compartmental organisation of the striatum.

Particular reference is made to the organisation of the connections of medium spiny type I neurons (MSI). Further information and evidence for these connections is contained in this chapter (see pages 36 - 38 ) and also in the results section of Chapter II (pages 58-92).



They were considered by these authors to be neurons because of their dendritic characteristics. Others, however, consider them as a type of glial cell (Chang et al, 1982; DiFiglia et al, 1976; Dimova et al, 1980; Fox et al, 1971/1972) as no investigators have demonstrated the presence of an axon, and because they are apparently only rarely seen in Golgi preparations.

In confirmation of a non-neuronal role for these cells, a study carried out by Dimova et al (1980) in which light and electron microscopic observations were correlated, failed to demonstrate the presence of synapses anywhere on their surface. As a result, they have been somewhat neglected in studies on striatal neuron morphology although their exact nature remains to be confirmed.

## 2. Compartmental organisation of the striatum

There is now a great deal of evidence to suggest that the striatum is organised into chemoarchitecturally distinct tissue compartments related to the disposition of several of the neurotransmitter-related compounds discussed earlier and to the organisation of striatal afferent and efferent connections. This organisation has also come to reflect the segregation of medium spiny neurons into either "patch" or "matrix" compartments which are mosaically arranged (Gerfen, 1984,1985). A diagram which summarises the current knowledge on this subject is shown in Figure 5.

### 2.1 Histochemical compartmentalisation

The histochemical compartmentalisation of the striatum has been demonstrated for a variety of neurotransmitters and related enzymes. The first observation of this phenomenon came with the

examination of AChE stained sections of human, cat and monkey striatum. These sections were marked by 300–600 $\mu$ m wide zones (i.e. patches) of low AChE activity which were in stark contrast to the AChE-rich background (Graybiel and Ragsdale, 1978a). These AChE-poor patches have also been observed in various other species (Graybiel and Ragsdale, 1978b) and appear to form highly connected labyrinths running through the striatum (Graybiel and Ragsdale, 1978a).

Subsequent studies have indicated that in cat and monkey these patches correspond to patches of high opiate-like immunoreactivity (Graybiel et al, 1980, 1981c; Graybiel and Chesselet, 1984) while in the rat they appear to correspond to regions of high opiate receptor density (Herkenham and Pert, 1981; Pert et al, 1975, 1976). Patches of SP-like immunoreactivity have also been reported (Penny et al, 1981; Graybiel et al, 1981c; Gerfen, 1984; Graybiel and Chesselet, 1984; Beckstead and Kersey, 1985, Beckstead, 1987) as have patches of DA histofluorescence (Olson et al, 1972; Tennyson et al, 1972), TH-immunoreactivity (Graybiel et al, 1987) neurotensin-like immunoreactivity (Goedert et al, 1983) somatostatin and neuropeptide Y (Chesselet and Graybiel, 1986; Gerfen, 1984; Graybiel et al, 1981c; Sandell et al, 1986), and GAD-immunoreactivity (Graybiel et al, 1981) although only some of these (in particular SP) have been related to AChE poor patches. The distributions of some receptor types as determined by autoradiography also display a similar arrangement e.g. opiate receptors (Herkenham and Pert, 1981; Pert et al, 1975, 1976), muscarinic receptors (Nastuk and Graybiel, 1985; Rotter et al, 1979), DA D<sub>2</sub> receptors (Joyce et al, 1985; Dubois and Scatton, 1985; Loopuijt et al, 1987) DA D<sub>1</sub> receptors

(Beckstead et al, 1988) and neurotensin receptors (Goedert et al, 1984).

There are strong indications that these arrangements are related to patterns laid down early in development. Both DA and AChE have been observed in small patches in fetal and neonatal striatum (Butcher and Marchand, 1978; Graybiel et al, 1981a,b; Graybiel and Ragsdale, 1980; Nobin and Bjorklund 1973; Olson et al, 1982; Ragsdale and Graybiel, 1979) while patches of high muscarinic and opiate receptor binding and high SP- and enkephalin immunoreactivity have also been reported (Kent et al, 1982; Rotter et al, 1979; Nastuk and Graybiel, 1985; Graybiel et al, 1981a, 1981c). With the onset of maturity the patches of high AChE activity, muscarinic receptor binding and high TH-immunoreactivity and DA fluorescence become obscured, leaving the poor AChE stain and only faint distinctions in muscarinic receptor binding, TH-immunoreactivity and DA fluorescence (Brand, 1980; Butcher and Hodge, 1976; Olson et al, 1972; Ragsdale and Graybiel, 1979; Rotter et al, 1979). However, some patches such as those immunoreactive for enkephalin remain unaltered through maturation which implies the maintenance of a three dimensional matrix throughout development.

## 2.2 Afferent and efferent connections of medium spiny neurons

Medium spiny neurons have been shown to receive differential input from cortex (Goldman-Rakic, 1982; Ragsdale and Graybiel, 1981; and see Chapter 2) and the nigrostriatal DA system (Gerfen et al, 1987; Wright and Arbuthnott, 1982; Beckstead, 1984). Direct serial-section comparisons of patchy input from cortex with histochemical compartments have been made in rat, cat and monkey



(see Chapter 2) and have been shown to correspond to either patch or matrix. Similarly, retrograde labelling studies have demonstrated that medium spiny neurons projecting to SN pars compacta (SNc) are, with their dendrites, located almost exclusively within the patch compartment and thus segregated from the matrix which coincides with medium spiny neurons which project to SN pars reticulata (SNr) (Gerfen et al, 1985). This organisation has been confirmed by other studies involving Golgi impregnation (Herkenham et al, 1984; Izzo et al, 1987) and intracellular labelling (Penny et al, 1984).

These observations have led to the suggestion that striatal patch and matrix compartments might provide segregated parallel input-output information processing channels (Gerfen 1984). However, this finding does not in itself prove that this is the case. It is more than likely that interactions between channels could occur, the nature and extent of which would depend on the structural organisation of patch and matrix tissue and the degree to which neurons respected their position within either compartment. In some respects it appears that segregation is maintained to a certain extent as the local axon collaterals of medium spiny neurons appear to remain restricted within the same location as their dendritic arbors (Wilson and Groves, 1980; Preston et al, 1980; Somogyi et al 1981a; Bishop et al, 1982). On the other hand it has been proposed that somatostatin and cholinergic interneurons could provide "cross-talk" between the two compartments. Both have perikarya and processes mainly in the matrix but individual processes and dendrites have been shown to cross boundaries (Gerfen, 1984, 1985; Graybiel et al, 1986; Chesselet and Graybiel, 1986; Graybiel et al, 1981c; Sandell et al, 1986). However, the role of

these interneurons in providing for intercommunication between patch and matrix compartments remains to be resolved.

Recent evidence now suggests that neuronal perikarya and dendrites may define the limits of patches identified by immunostaining for SP or by AChE histochemistry. Bolam et al (1988) found that medium spiny neurons lying within patches and close to their boundaries were of two types: those whose dendritic arbors were not influenced by the boundaries and a second group whose dendritic arbors were markedly influenced such that they exhibited abrupt changes in their course to avoid crossing the boundaries. A similar situation occurred in the matrix compartment.

A recent study which examined the three-dimensional distribution of enkephalin-rich compartments in the cat striatum (Groves et al, 1988) showed that these patches formed highly interconnected structures. Finger-like processes and crossbridges, which were much in evidence, were suggested to provide for orderly interaction between patch and matrix compartments. In contrast, however, was the finding by Penny et al (1988) that dendrites and local axon collaterals of enkephalin-immunoreactive neurons were confined by the boundaries of enkephalin-rich patches, but that dendrites of neurons with similar characteristics to large striatonigral type II neurons did extend across the compartmental boundaries. Thus it appears that total segregation of the two compartments does not occur and that different populations of medium spiny neurons underlie the segregation of information in distinct compartments and communication between compartments.

### 3. Aims of the investigation

The preceding literature review has described some of the large amount of knowledge concerning the anatomy of the striatum. In the next chapter, the inputs from specific cortical areas to striatum will be examined in relation to its compartmentalisation, while Chapter 3 investigates this phenomenon electrophysiologically in an 'in vitro' slice preparation. The ability of host cortex to innervate striatal grafts as demonstrated electrophysiologically, is dealt with in Chapter 4. The controversy surrounding the effects of DA on striatal neuron activity and corticostriatal transmission is examined in Chapter 5 with a view to clarifying the role of this transmitter within the striatum.

## CHAPTER 2

### An Anatomical Study of the Corticostriatal Pathway

## INTRODUCTION

The first evidence for the existence of a projection from the cerebral cortex to the striatum came from Luys (1865). His functional conceptualisation of such a projection was as the exclusive route from cortex to thalamus via the striatum. Similar work carried out by Cajal (1911) provided evidence to suggest that this pathway was composed of axon collaterals from corticofugal fibres in the internal capsule.

Most other early authors affirmed the existence of a pathway from cortex to the basal ganglia, although they differed in their opinions as to which of the nuclei received inputs. In particular, the possible existence of a striatal input was the focus of many contradictory reports. Most of these stemmed from the use of the Marchi method (1866) for the visualisation of degeneration in myelinated axons, and possibly from the use of a wide variety of experimental animals. Those studies which confirmed the findings of Luys (1865) and Cajal (1911) were carried out in cats (Poljak, 1927; Bianchi, 1914) rabbits (Coenen, 1929) and monkeys (Mettler, 1935, 1942; Kennard and Fulton, 1949). Conversely, other experiments carried out in monkeys (Levine 1936; Wilson, 1914; Verhaart and Kennard, 1940), cats (Le Gros Clark and Boggan, 1933) and rats (Kreig, 1947; Le Gros Clark and Boggan, 1933) all suggested that the striatum was independent of cortical input.

The study carried out by Wilson (1914) was one such report which came down against a corticostriatal pathway. The evidence presented did imply, however, that a few small fasciculi might be crossing into the putamen from the contralateral side, but Wilson suggested

that they were fibres of passage, a view which was widely accepted at the time. Several decades later, in an extensive review of the subject, Mettler (1942) claimed that Wilson's evidence was flimsy and based upon capsular lesions, not cortical removals. He concluded that the striatum was indeed under some degree of cortical control, an opinion which had received much favour in the intervening years after Wilson's report.

Further evidence in favour of the pathway was obtained from electrophysiological experiments (Dusser de Barenne et al., 1935, 1942) in which a technique called physiological neuronography was used to examine the "functional organisation" of the sensory cortex of monkeys. This essentially involved the recording of the electrical activity produced by local cortical stimulation evoked by the application of filter paper strips soaked in strychnine solution. The distribution of strychnine-induced spikes was taken to represent the regions to which that particular area was connected and with which it was in functional relationship.

During the mapping of cortico-cortical connections, Dusser de Barenne et al. discovered that when a narrow strip of cortex at the margin of area 4 was stimulated electrically, mechanically or chemically, a transient diminution of electrical activity was elicited. This decreased activity extended gradually from the strip region to area 4 and subsequently to the rest of cortex. They named this area 4S (S for suppression), an area which had been independently discovered by Hines (1937) to cause relaxation of lasting muscle contractions. The suppression elicited from stimulation of this area was later found to be prevented by undercutting the cortex. It was suspected that the suppressive

effects were mediated by a cortico-thalamo-cortical pathway, but it was later found to be prevented by a striatal lesion. Further investigations led to the discovery of other suppression areas - 2S, 8S, 19S - which projected to the caudate nucleus while areas 4 and 6 projected to the putamen in monkeys. Similar findings were obtained by Garol (1942) using the same technique on cat cortex.

In an attempt to confirm the findings of Dusser de Barenne et al., Verhaart and Kennard (1940) looked for degeneration in the monkey caudate after lesioning areas 4, 6 and 4S. They did not find any but suggested that this was due to the demyelination of corticofugal fibres on entering the striatum. Glees (1944) shared this opinion after using a modified Marchi technique to demonstrate corticostriatal fibres in cats and rabbits. However, using another technique which involved the utilisation of silver preparations he showed that cortical areas 2S, 3S and 8S in cats were connected to the striatum and thought that these fibres arose as collaterals from corticofugal fibres, thus essentially confirming the work of Dusser de Barenne et al.

Later work, however, refuted the claims of Dusser de Barenne et al. (Druckman, 1952; Gellhorn, 1947; Leao 1944; 1947). Criticism came from Druckman (1952) who commented on the small number of lesions made and on the variability of the monkey cortical surface. He was also unable to confirm the existence of suppressor areas from his own experiments.

Leao (1944; 1947) in his work on rabbits, observed a diminution in spontaneous electrical activity of the cortex which could be elicited from and could spread to almost any point on the cortex. He referred to this phenomenon as "spreading depression" and

reported that its time course was very similar to the effects described by Dusser de Barenne et al. This lead was pursued further by Sloan and Jasper (1950) who found identical effects on the encephalogram were produced by the application of strychnine to either suppressor or non-suppressor areas. They also showed that these effects were present after undercutting the cortex, which refuted the claims that subcortical pathways were required for suppression.

Despite the evidence against the ideas that the strip areas and motor areas 4 and 6 gave rise to the corticostriatal afferents, this concept was widely believed to be the case (Jung and Hassler 1960). However, due to the advent of better histological methods these ideas were largely dispelled, such that a number of studies based on the Nauta and Gyax method for silver impregnation (Nauta and Gyax, 1954) eventually led to the establishment of sound experimental evidence for the pathway and also demonstrated a topography which was consistent among several species (Webster, 1961; 1965; Carman et al., 1963; Martin and Hamel, 1967; Kemp and Powell, 1970; Whitlock and Nauta, 1956; Devito and Smith, 1964; Johnson et al., 1968; Domesick, 1969).

Webster's experiments (1961) employed a variety of histological methods to visualise degenerating fibres in rats and cats. A modified Marchi method (Swank and Davenport, 1935) proved unsuccessful in revealing degeneration of fibres within the striatum after large frontal cortex lesions. However, using the Nauta and Gyax method (1954) degenerating axons were observed to pass through the white matter into the internal capsule, terminating within the striatum. Webster also showed that the projection was organised



topographically in both the anterior-posterior and medio-lateral planes and was derived from widespread areas of cortex.

Subsequent studies on rabbits (Carman et al., 1963; 1965), cats (Webster, 1965) and monkeys (Devito and Smith, 1964; Johnson et al., 1968; Kemp and Powell, 1970) confirmed these findings. They also demonstrated that all cortical areas project largely to proximal or nearby parts of the striatum, with a considerable amount of overlap occurring in their terminal fields (Kemp and Powell, 1970). The striatum was shown to receive projections from motor and primary sensory cortical areas. The most dense projection came from the sensorimotor cortex and the smallest from the visual cortex (Kemp and Powell, 1970).

Since the use of autoradiographic tracing methods it is now clear that silver impregnation methods for visualising degenerating axons failed to reveal certain axon terminals and consequently underestimated the total projection from a given population of neurons. Additionally, the use of autoradiography has revealed that the terminals form mosaic-like patterns within the striatum, a phenomenon which was mentioned by Kemp and Powell (1971).

The autoradiographic technique essentially involves the injection of tritiated amino acids into the cortex. These amino acids are incorporated into proteins by the neurons and transported to the axon terminals where they can be visualised by autoradiography. Consequently, injections made into the rat somatosensory projection cortex (Beckstead 1979), rat medial agranular cortex (Reep et al., 1987), monkey somatosensory cortex (Jones et al., 1977), monkey prefrontal cortex (Goldman and Nauta, 1977; Goldman-Rakic, 1982), monkey temporal cortex (Ragsdale and

Graybiel, 1981; 1984), monkey precentral cortex (Kunzle, 1975; 1977; 1978), monkey temporal cortex (Van Hoesen et al., 1981), monkey frontal eyefields (Stanton et al., 1988), primary and supplementary cortex in the dog (Tanaka et al., 1979; 1981) all show that circumscribed areas of cortex project throughout all anterior-posterior subdivisions. Similarly, motor and premotor cortex have extensive anterior-posterior fields in the monkey putamen (Kunzle 1975; 1977; 1978; Jones et al., 1977).

These studies have also demonstrated the presence of clusters of silver grains on autoradiographs of the striatum. The patch-like appearance of the terminal fields were surrounded by areas in which the grain density did not exceed background. Occasionally the clusters were seen to surround grain free cores. This pattern of distribution has been shown to be more sharply defined in newborn than in older monkeys (Goldman and Nauta, 1977) such that with increasing age, some of the grain-free cores become filled and the clusters become much more diffuse. Goldman and Nauta went on to suggest that the striatum is organised as an anatomical and functional mosaic rather than a homogeneous structure as was previously thought.

#### Distribution of cortical inputs to striatum

Recent anatomical tracing studies have provided some information on the convergence of projections arising from cytoarchitectonically distinct cortical areas (Yeterian and Van Hoesen, 1978; Pearson et al., 1983). Yeterian and Van Hoesen (1978), in their study on several corticostriatal connections not only confirmed the earlier findings (Kemp and Powell, 1970; Goldman and Nauta, 1977)

that there was a certain degree of overlap between the terminal fields of separate cortical areas, but also that these cortical areas were often reciprocally connected via cortico-cortical projections, a finding which was confirmed by Tanaka (1983).

Although these findings were widely interpreted to represent a general principle of organisation for corticostriatal circuitry, a relatively small number of reciprocally connected cortical areas were examined. In a report by Selemon and Goldman-Rakic (1985), criticism was made of the method of analysis used by Yeterian and Van Hoesen. This had involved the use of an inter-animal comparison of terminal fields, such that exact determination of the degree of overlap between cortical projections would be difficult. Consequently, Selemon and Goldman-Rakic re-examined the topography and degree of convergence or segregation in corticostriatal afferents originating from functionally distinct cortical areas by the use of double labelling techniques. It was found that there was an interdigitation of corticostriatal terminal fields reminiscent of cortical columns (Hubel et al., 1974), rather than an overlap. They concluded that the idea of Yeterian and Van Hoesen was not a general principle of organisation in the corticostriatal pathway and speculated that, in the rhesus monkey, the cortically receptive striatum, could be further differentiated into longitudinal functional domains each of which receives a unique subset of cortical projections.

While most studies established that the cortical projections terminated in a mosaic-like pattern within the striatum, it was unclear whether this pattern was solely the result of the topography of the cortical projection on to the striatum or whether cortical

fibres were obeying some plan of organisation intrinsic to the striatum itself. The possibility of such internal compartmentalisation of striatum was first raised in the studies of cellular architecture and inhomogeneities in AChE staining in cat, monkey and human brain (Graybiel and Ragsdale, 1978). This and subsequent studies revealed that the striatum could be compartmentalised such that the compartments consisted of AChE-poor, SP-immunoreactive and  $\mu$ -opiate receptor-rich regions called patches or striosomes and a surrounding AChE-rich subdivision, the matrix. (See Ch 1 for more information and references).

In studies carried out on cats and monkeys Ragsdale and Graybiel (1981; 1984) compared the patterns of termination of frontal cortico-striatal fibres with patterns of striatal acetylcholinesterase activity. The clustering patterns of corticostriatal terminals were found to correspond to a large extent to the inhomogeneities in enzyme activity identified by acetylthiocholine staining methods.

Subsequent studies on inputs from other cortical areas confirmed the initial observations of Ragsdale and Graybiel (Gerfen, 1984; Donoghue and Herkenham, 1983; 1986). Gerfen (1984), using the sensitive PhAL anterograde axonal tracing method (Gerfen and Sawchenko, 1984) found that prelimbic cortex in the rat projected predominantly to striatal SP-immunoreactive patches, while Donoghue and Herkenham (1983), using autoradiography, showed that the matrix received inputs from sensory and motor cortex. The observation that medium spiny neurons within these patches projected to SNc while matrix neurons projected to SNr, with an intrinsic striatal somatostatin-immunoreactive system linking the two, led Gerfen

(1984) to suggest that the striatal compartments were functionally distinct and interactive parallel input-output processing channels overlaid on the more classically recognised topographically ordered dorsal (non-limbic) and ventral (limbic) segregation of striatal connectivity and function (Heimer et al., 1975; Mogensen et al., 1983; Kelley et al., 1982; Graybiel and Ragsdale, 1978).

A subsequent study by Donoghue and Herkenham (1986) expanded on these initial observations, by mapping corticostriatal projections from other cortical fields on to marked striatal compartments using axonal transport of [ $^3\text{H}$ ] amino acids and receptor localisation techniques in the rat brain. They confirmed that prelimbic cortex projects to patches, and also showed that agranular motor and cingulate cortices project preferentially to matrix in both ipsilateral and contralateral striatum. Somatosensory and visual cortices were also shown to project to matrix but only in the ipsilateral striatum. Each matrix input appeared to occupy a characteristic striatal district while patches were distributed throughout the striatum, thus allowing for prelimbic cortex interactions with input from other cortical areas.

Additional patterns of corticostriatal projections have still to be revealed through studies of other cortical fields. The question also remains of whether any cortical areas provide a significant input to both patch and matrix compartments in the striatum.

#### Bilateral projection

A bilateral corticostriatal projection was first demonstrated in the degeneration experiments of the early 1960s (Carman et al., 1963; Webster, 1965). In these experiments the contralateral

striatum was seen to have undergone degeneration after lesions in the sensorimotor cortex alone. Consequently it was thought that in monkeys the projection was confined to this area (Jones et al., 1977; Kemp and Powell, 1970).

Later experiments however, revealed that other cortical regions contribute to this bilateral projection in monkeys. For instance, several studies (Goldman and Nauta 1977; Fallon and Ziegler 1979; Leichnetz, 1981) have indicated that the prefrontal cortex is also involved, while Kunzle (1977; 1978) has demonstrated a primary somatosensory cortical component. Other work has also examined the bilateral projection of small areas within the sensorimotor cortex; for example that of Miyata and Sasaki (1984) who examined HRP-stained material from the mesial part of area 6.

A bilateral projection from several cortical regions has also been found in the rat (Beckstead, 1979; Faull et al., 1984; Veening et al., 1980; Reep et al., 1987) cat (Garcia-Rill et al., 1979; Olea 1980; Battaglini et al., 1982; Royce, 1982; Fisher et al., 1984; Kubuzano et al., 1986), dog (Tanaka et al., 1981, 1979) and raccoon (Tanaka and Sakai, 1985), and has been observed to cross the midline in the corpus callosum. Martin and Hamel (1967) also found that in the opossum, the bilateral projection arises from frontal, orbital and parietal regions but crosses in the anterior commissure.

The question arose as to whether two populations of cortical neurons are involved, one projecting to ipsilateral striatum, the other to the contralateral side, or whether the contralateral projections arise from collaterals of the ipsilateral projecting neurons. Some studies involving retrograde labelling with HRP demonstrated that corticostriatal neurons projecting to

contralateral striatum are localised in the same areas and sublaminae as those projecting to ipsilateral striatum (Jones et al., 1977; Oka, 1980; Royce, 1982; Wise and Jones 1977; Kemp and Powell, 1970; Kunzle, 1975; 1978; Fallon and Ziegler, 1979; Tanaka et al., 1981; Fisher et al, 1984; Tanaka and Sakai, 1985).

The ability to determine whether these two populations overlapped came partly from the introduction of double labelling techniques (Kuypers et al., 1977). Studies carried out in cats (Fisher et al., 1984) and rats (McGeorge and Faull, 1987) have demonstrated the existence of corticostriatal neurons which send collaterals to both striata. This number is, however, very small; McGeorge and Faull (1987) showed that only 13% of corticostriatal motor and sensory cortex neurons were double labelled after injection of WGA-HRP into one striatum and [ $^{125}$ Iodine]-WGA into the other.

The existence of such branched neurons in the rat prefrontal cortex has also been suggested by several electrophysiological experiments. Wilson (1986) examined the characteristics of postsynaptic potentials evoked in striatal neurons following stimulation of ipsilateral and contralateral cortex. He found that identical excitatory postynaptic potentials (EPSPs) in striatal neurons on the contralateral side were evoked after stimulation of the ipsilateral cortex or striatum and concluded that these EPSPs were due to activity in a single population of cortical neurons since either could be occluded by a previous stimulation applied to the other.

In a further study by Wilson (1987) in which Wheatgerm Agglutinin conjugated with HRP (WGA-HRP) was used as a retrograde



tracer and coupled with electrophysiological recording, his previous observations were essentially confirmed. Additional evidence was provided to suggest that there was an overlap in the population of crossed corticostriatal neurons and those projecting to contralateral cortex. Ten out of 32 crossed corticostriatal neurons studied also responded antidromically to stimulation of the corresponding region of the contralateral cortex. The axons of intracellularly stained examples of these neurons were also found to have one or more branches which also gave rise to a projection to ipsilateral striatum and/or cortex.

A similar study was carried out by Ferino et al (1987) in the rat medial prefrontal cortex. The antidromic activation technique was used to identify the cortical neurons which innervate the ipsilateral and contralateral striata as well as contralateral homotypic cortical areas. Of all the neurons identified in this way, approximately 38% were found to be antidromically driven from at least one of the projection areas, while the reciprocal collision test provided evidence for the existence of branched axons for 35% of the antidromically activated neurons. It was also observed that 46% of cortical neurons which were found to project to the homotypic contralateral area also projected to either ipsilateral or to ipsi- and contralateral striatum.

Thus it appears that there is a large number of cortical neurons with divergent axon collaterals which not only project bilaterally to the striatum but also to the homotypic contralateral cortex. The functional significance of this phenomenon remains to be resolved, but various workers (Ferino et al., 1987; Royce, 1984; Fisher et al., 1984) have speculated that the crossed corticostriatal





projection may be a major contributor to the coordination of activity in the striatum of both hemispheres.

### Cells of Origin

The introduction of retrograde axonal tracing using the enzyme Horseradish peroxidase (HRP) (Nauta et al., 1974; LaVail and LaVail, 1972) made it possible to visualise the cells afferent to a particular area. However, in early studies using this technique (Nauta et al., 1974; Jones and Leavitt, 1974) the corticostriatal pathway could not be demonstrated. This failure has since been considered to be due to low intensity of the reaction product obtained and the lack of a dark field optic system for visualisation of the labelled neurons (Jones et al., 1977). Since then, numerous successful HRP studies have been carried out in the rat (Wise and Jones, 1977; Hedreen, 1977; Donoghue and Kitai, 1981; Veening et al., 1980; Schwab et al., 1977), cat (Kitai et al., 1976; Oka, 1980; Royce, 1982; Kubozano et al., 1986), monkey (Jones and Wise, 1977; Arikuni and Kubota, 1986) and the dog (Tanaka et al., 1979; 1981; Tanaka, 1987a, 1987b).

In the monkey and the rat the neurons are medium sized (14-16 $\mu$ m in diameter)(Jones and Wise, 1977) and localised mainly in lamina V (Landry et al., 1984; Wise and Jones, 1977; Donoghue and Kitai, 1981). Double labelling experiments and WGA-HRP tracing techniques have confirmed this (McGeorge and Faull, 1987; Wilson, 1987; Ferino et al., 1987), although they report that the neurons tend to be more widespread, being distributed also in laminae III and VI, but to a much lesser degree than in lamina V. Tanaka (1987a, 1987b) also found this to be the case for prefrontal cortex neurons projecting to striatum in the dog.

Initial findings in the cat (Kitai et al., 1976) were that the projection comes mainly from layer III and subsequent studies have confirmed this (Oka, 1980; Royce, 1982; Fisher et al., 1984; 1986; Kubozano et al., 1986). Oka (1980) extended Kitai's work, and suggested that two corticostriatal projections exist, one direct projection from small pyramidal neurons in lamina III and the other from larger pyramidal neurons in lamina V projecting to lower structures such as the brain stem or spinal cord.

A great deal of debate has since surrounded the possibility that a component of the projection is from collaterals of corticofugal fibres. From electrophysiological experiments, Kitai et al (1976) concluded that corticostriatal and corticospinal pathways were completely independent in the cat. Similarly, other studies carried out in the cat (Jinnai and Matsuda, 1979; Oka, 1980) found that there were two populations of neurons, one of which projected only to striatum while the other projected to structures below the level of the cerebral peduncle. Studies in the rat (Wise and Jones, 1977) and monkey (Jones et al., 1977) also suggested that the two systems were independent, this view being based on the relative sizes and distributions of the two identified neuron populations.

More recent evidence suggests that collaterals from brain stem and/or spinal cord projections do terminate in the striatum. Donoghue and Kitai (1981) employed antidromic activation and retrograde HRP labelling to show that this is the case in the somatosensory motor cortex of the rat. They observed that only 25% of the neurons that were found to project to the brain stem had fine collateral branches in the striatum and were located in lamina Vb. Similar studies carried out by Wilson et al. (1982), Wilson (1986)

and Landry et al (1984) in rat have confirmed that brain stem projecting neurons can contribute axon collaterals to the striatum in the course of their passage in the internal capsule, and that these collaterals make excitatory synaptic contacts on striatal spiny neurons.

An elegant double labelling study in the cat (Royce, 1983) offers direct confirmation that axon collaterals are involved. The fluorescent tracers, Evans Blue and Fast Blue were injected into the striatum and the centromedian parafascicular thalamic complex respectively. Double labelled neurons were observed in laminae V and VI of specific cortical regions but were relatively few in number compared to the very numerous corticostriatal neurons (labelled singly with Evans Blue) located in laminae II, III, V and VI. This work therefore essentially confirms the suggestion of Oka (1980) that two corticostriatal projections exist, one from lamina III, the other from lamina V.

This collateral pathway does however lack several of the known characteristics of the corticostriatal pathway as a whole and evidence outlined above suggests that the direct corticostriatal pathway contributes the bulk of the projection. Wilson (1987) was able to separate these two populations of cells in the rat by examining those cortical areas with bilateral projections to the striatum, which have been shown to be free of axonal branches that descend to the brain stem (Catsman-Berrovets et al., 1980; Miller, 1975). This relatively pure population of crossed corticostriatal axons were identified by antidromic stimulation from the contralateral striatum and by the injection of WGA-HRP. They were found to be medium sized and situated in the superficial part of

lamina V and deep III, whereas brain stem projecting cortical neurons collected in the same animals had larger somata which were usually located deep in lamina V. The results therefore confirmed the earlier studies of Donoghue and Kitai (1981), Wilson (1986) and Landry et al (1984) and are directly comparable to those obtained in the cat (e.g. Oka 1980; Royce, 1983).

As mentioned earlier additional patterns of corticostriatal projections have still to be revealed. Therefore, in this section of the thesis, the organisation of corticofugal projections of the rat medial prefrontal cortex was examined with the PhAL anterograde tracing method. The distribution of corticostriatal efferents in the striatal patch and matrix compartments, as distinguished by staining for calcium binding protein (CaBP)-immunoreactivity (Gerfen et al., 1985), are described in terms of the cortical area of origin and compared with patterns of cortico-cortical and cortico-thalamic projections.

## METHODS

### Animals

Female albino rats (250g) were used throughout the experiments.

### PhAL injections

Injections of PhAL and subsequent histological methods were carried out as described by Gerfen and Sawchenko (1984).

### Immunohistochemical localisation of CaBP

CaBP-immunoreactivity was stained for in the brain sections using the method of Gerfen (1985).

### Microscopic analysis

PhAL stained sections were examined using an Olympus microscope. Injection sites were examined and the location of filled neurons confirmed by the thalamic inputs visible in the sections. The pattern of striatal staining was also examined and compared to the pattern of CaBP staining visible in adjacent sections. A direct comparison was made possible by the use of a camera lucida attachment so that the image of an adjacent CaBP striatal section could be superimposed upon a drawing of a PhAL stained section. All other PhAL labelled areas were noted in order to determine if any correlation existed between staining patterns observed in different cases.

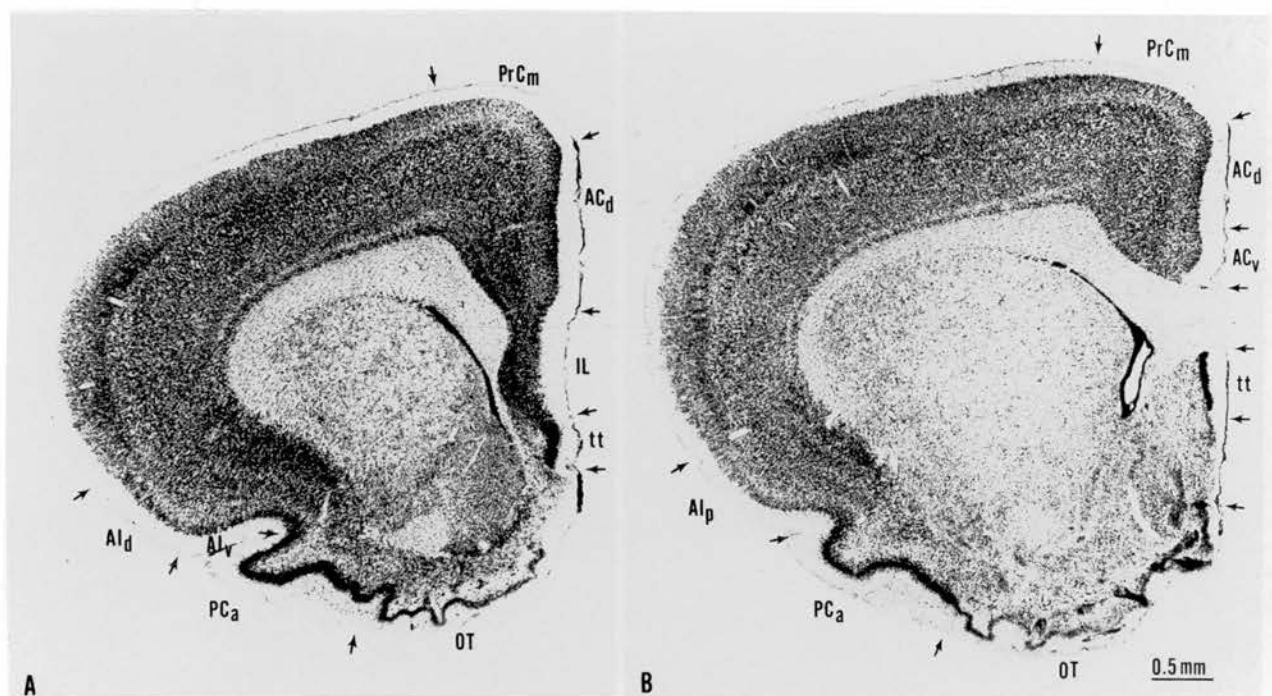
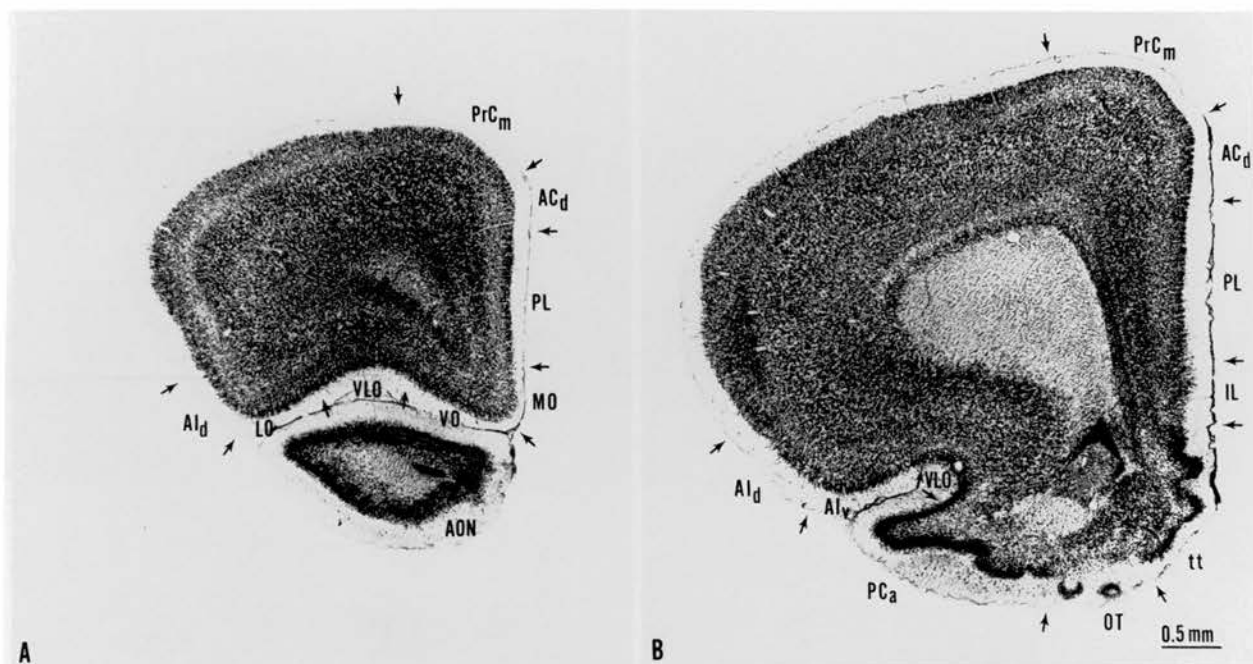


FIGURE 6: Subdivisions of the prefrontal cortex.  
Photomicrographs of thionin-stained coronal section (50 $\mu$ m)  
through the frontal cortex of the rat.  
Arrows mark the boundaries between cortical areas

Taken from Krettek and Price (1977).

## RESULTS

The data presented in this section is derived from material obtained from 31 rats injected with PhAL.

### Injection sites

Iontophoretic injections of PhAL resulted in a very characteristic labelling pattern at the injection site in each case. Usually a small brown-stained area was present which was due to residual reaction product. Within this area and surrounding it, densely stained perikarya were usually visible with often distinct labelling of dendrites and dendritic specialisations.

With most cases examined the area of origin of labelled projections was fairly easy to determine, but in those cases where the injection site covered more than one cortical area or was situated close to the "boundary" between two areas, it was often difficult to define where one area ended and the next began. This situation was often made more difficult by the deposition of a dark reaction product in the areas around the labelled perikarya.

One aid to the identification of each cortical area comes from the work of Krettek and Price (1977) who mapped out the prefrontal areas of the rat in terms of their lamination (Figure 6). Using autoradiographic tracing they found that infralimbic cortex has relatively indistinct lamination so that laminae I and II tend to fuse together somewhat. In contrast, prelimbic cortex has well-defined lamination with a relatively homogeneous layer V and a tightly packed bilaminar layer VI. Anterior cingulate cortex, on the other hand, is easily distinguished from prelimbic cortex by its



layer V which is conspicuously broader and less densely packed. In this study however, it was found that when observing filled cortical cells under dark field optics, laminae and consequently cortical areas were often difficult to identify. Therefore, a "back-up" means of identification comes from the examination of corticothalamic and often corticocortical projections.

Several studies have indicated that the prefrontal cortex provides a large input to and receives a considerably large reciprocal connection from the thalamus, in particular the mediodorsal thalamic nucleus (Figure 7) (Beckstead, 1979; Krettek and Price, 1977; Leonard, 1969; 1972; Siegel et al., 1977; Groenewegen, 1988; Room et al, 1985; Rose and Woolsey, 1948; Cornwall and Phillipson, 1988). Definition of the prefrontal cortex by its thalamic afferents rather than its cytoarchitecture has, in fact, been suggested by several authors (Rose and Woolsey 1948; Kolb, 1984), and this system will be utilised in this study.

Dense labelling of callosal fibres was observed in most cases as were fibres within corticostriatal fibre bundles which had been cut in cross section in these samples. They could be distinguished from striatal patches as they tended to be smaller and much more uniform in shape.

The criteria for subdividing the cases were as follows:

- (i) Neurons labelled in prelimbic cortex only.
- (ii) Neurons labelled in infralimbic cortex only.
- (iii) Neurons labelled in both pre- and infralimbic areas.
- (iv) Neurons labelled in both prelimbic and medial orbital areas.
- (v) Neurons labelled in anterior cingulate cortex only.
- (vi) Neurons labelled in medial agranular cortex only.



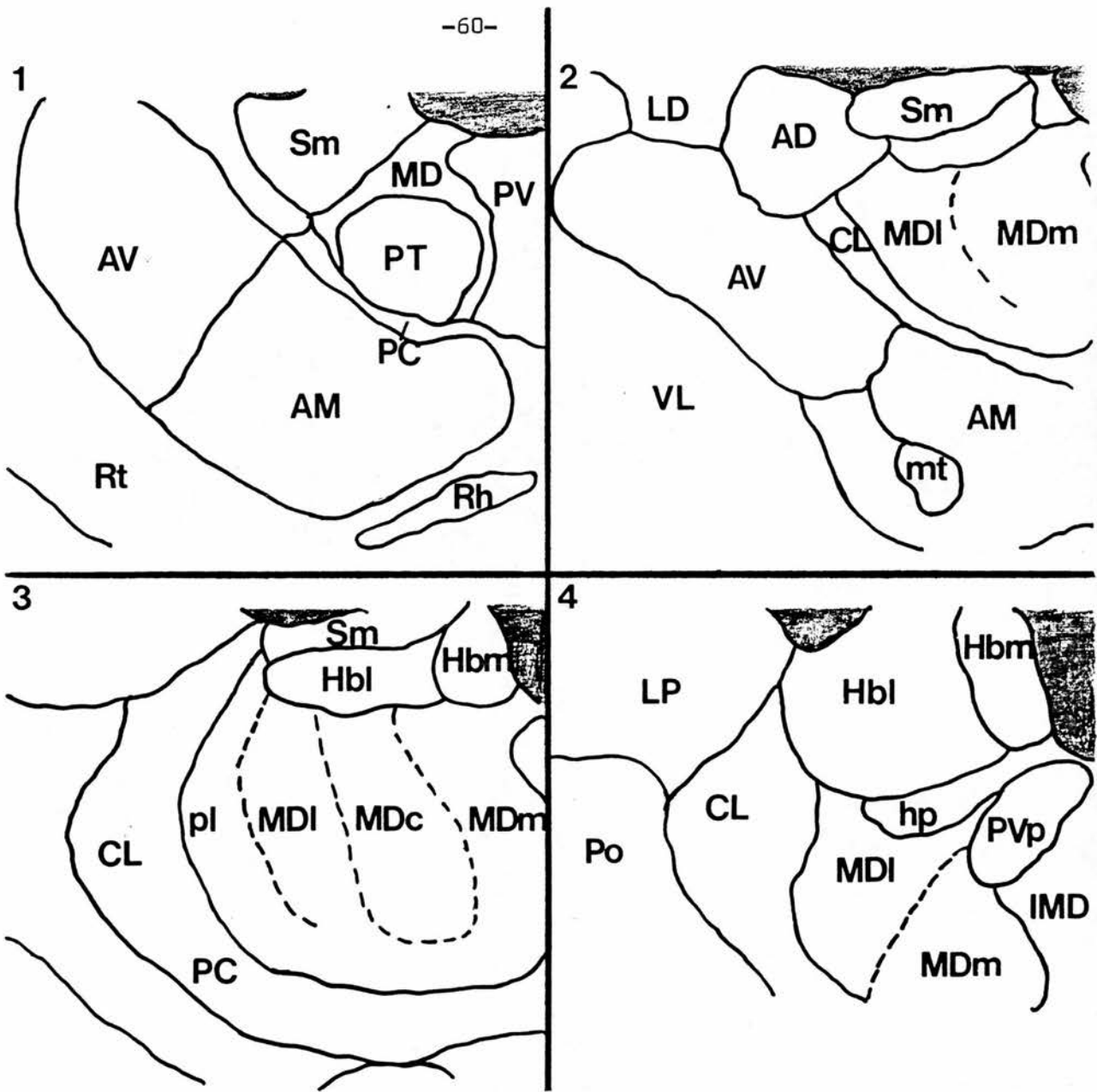


FIGURE 7: Subdivisions of the mediodorsal thalamic nucleus and surrounding structures.

Redrawn from Groenewegen (1988)

Abbreviations:

AD	Anterodorsal thalamic nucleus	MD	Mediodorsal thalamic nucleus medial division
AM	Anteromedial thalamic nucleus	mt	mammillothalamic tract
AV	Anteroventral thalamic nucleus	PC	paracentral thalamic nucleus
CL	Centrolateral thalamic nucleus	pl	Paralamellar division mediodorsal thalamic nucleus
Hbl	lateral habenular nucleus	Po	posterior thalamic nuclear group
Hbm	medial habenular nucleus	PT	Paratenial thalamic nucleus
hph	habenular interpeduncular tract	PV	paraventricular thalamic nucleus
IMD	intermediodorsal thalamic nucleus	PVP	paraventricular thalamic nucleus, posterior part
LD	laterodorsal thalamic nucleus	Rh	rhomboid thalamic nucleus
LP	lateral posterior thalamic	Rt	reticular thalamic nucleus
MD	Mediodorsal thalamic nucleus	SM	nucleus of the stria medullaris
MDC	Mediodorsal thalamic nucleus, central division	VI	ventrolateral thalamic nucleus
MDI	Mediodorsal thalamic nucleus, lateral division		

- (vii) Neurons labelled in prelimbic and anterior cingulate areas.
- (viii) Neurons labelled in prelimbic, infralimbic and anterior cingulate cortex.

Examination of the location of the labelled perikarya with respect to cortical laminae revealed that those mainly in layer V were labelled with PhAL. However, several cases displayed PhAL-stained perikarya in more superficial layers, especially layer III (deep portion) (see Figures 13,15,21,23).

### Striatal labelling

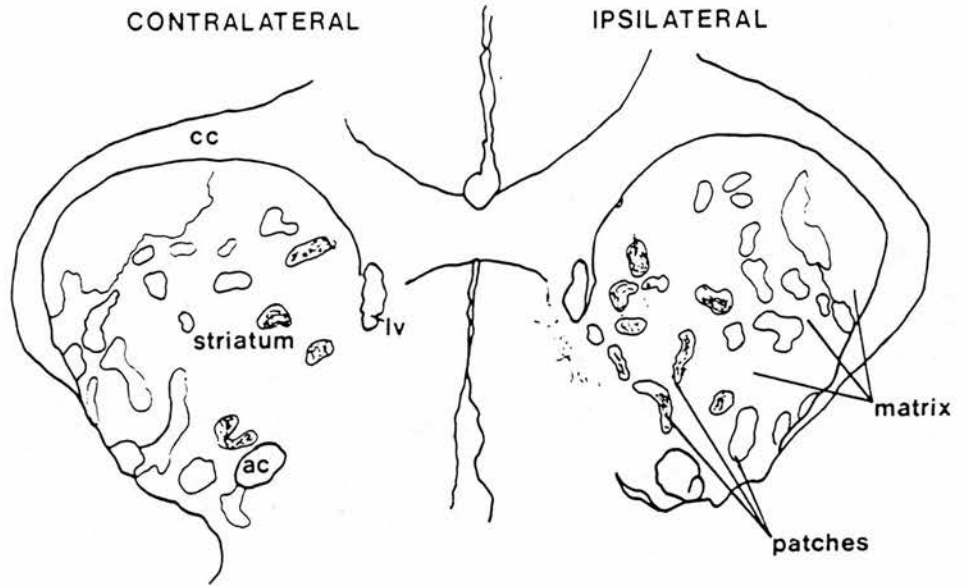
Almost all the cases showed clear PhAL labelling in ipsilateral and contralateral striatum, although the former area generally presented more intense staining (Figure 8). This labelling either had a very "grainy" appearance, indicating the presence of terminals, or appeared fibre-like to a lesser degree. Besides terminals being very clear, smaller varicosities were also observed on fibres in some of the better sections.

One case, (RM-12) displayed very little labelling of terminals and fibres and was found to have a very poor injection site. Consequently this case was discarded from the analysis.

Closer examination of ipsilateral corticostriatal projections revealed the presence of three distinct types of labelling pattern:

- (a) Predominant labelling in patches which were coincident with patches observed in the adjacent sections stained for CaBP-immunoreactivity. These patches varied in diameter (100-450 $\mu$ m) and were fairly irregular in shape. They occurred mainly in dorsomedial/medial/ventromedial regions, mainly in the rostral half of the striatum.

A. RM-2



B. RM-18

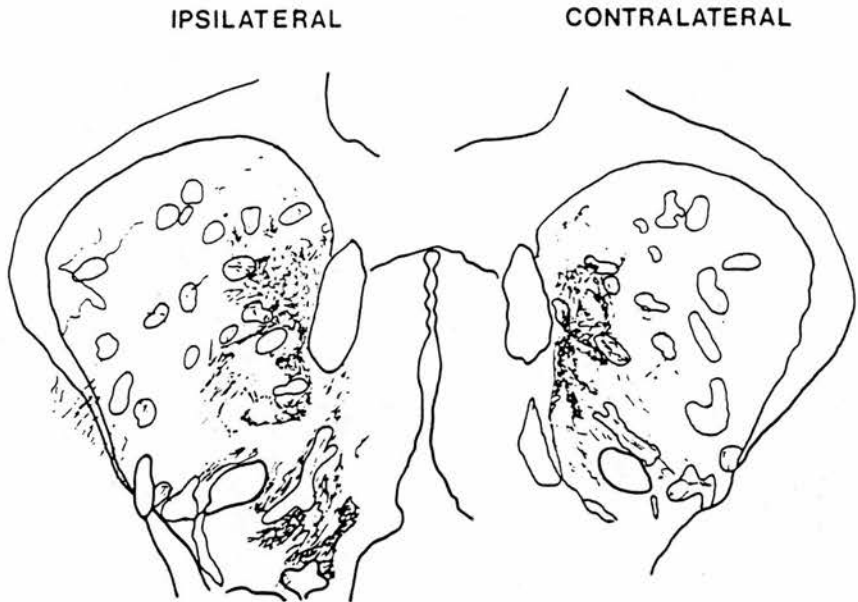


FIGURE 8: Comparison of PhAL labelling in ipsilateral and contralateral striata.

Two examples, RM-2 and RM-18, which demonstrate clearly that contralateral striatal PhAL labelling is less intense than that in ipsilateral striatum.

Note that RM-2 received an injection of PhAL into the left hemisphere. All other animals received right-side injections.

Abbreviations:

ac anterior commissure  
cc corpus callosum  
lv lateral ventricle

ABBREVIATIONS :

P	: patches
M	: matrix
pl	: paralamellar
l	: lateral
m	: medial
r	: rostral
CM	: centromedial thalamic nucleus
VL	: ventrolateral thalamic nucleus
CL	: centrolateral thalamic nucleus
PT	: paratenial thalamic nucleus
VM	: ventromedial thalamic nucleus
AM	: anteromedial thalamic nucleus
AD	: anterodorsal thalamic nucleus
AV	: anterodorsal thalamic nucleus
PC	: paracentral thalamic nucleus
FrPaSS	: frontoparietal cortex , somatosensory area
FrPaM	: frontoparietal cortex , motor area

TABLE 3 : SUMMARY OF RESULTS OF PHAL LABELLING

INJECTION SITE	CASE No	LOCATION OF INJECTED NEURONS (CORTICAL LAYERS)	STRIATAL LABELLING	MEDIODORSAL THALAMIC NUCLEUS LABELLING	OTHER THALAMIC LABELLING	CONTRALATERAL HOMOLOGOUS CORTICAL LABELLING (IN LAYERS)	LABELLING IN OTHER CORTICAL AREAS	LABELLING IN OTHER BRAIN REGIONS (MAIN AREAS)
(i) PRELIMBIC	CSI-3	V, VI	P	pl=I	CM, PT	deep >> superficial	anterior/posterior cingulate retrosplenial	corpus callosum , nucleus accumbens , claustrum
	CSI-4	V	P	pl=I	CM, CL	deep >> superficial	anterior/posterior cingulate	
	CSI-7	V, VI	P, M	I	CM, CL	deep fibres, superficial terminals	-	
(ii) INFRALIMBIC	RM-2	V	P	m	PT	mainly deep layers	anterior/posterior cingulate	corpus callosum , nucleus accumbens , claustrum , central gray area , bed nucleus of the anterior commissure
	RM-6	V	P	r (m=I)	PT	deep layers only	anterior/posterior cingulate	
	RM-7	V, VI	P	m	PT	deep >> superficial	temporal/entorhinal	
	RM-3	V	P, M	I	PT, VM	superficial $\geq$ deep	anterior/posterior cingulate , retrosplenial , temporal/entorhinal	
(iii) INFRALIMBIC/ PRELIMBIC	RM-9	V, VI, deep III	dense + "holes"	r/m	PT, CM	superficial $\geq$ deep	anterior/posterior cingulate , temporal/entorhinal	corpus callosum , nucleus accumbens , internal capsule , central gray area , zona incerta , globus pallidus , claustrum
	RM-10	V, VI, up to II	P, M	r	CM	superficial = deep	anterior/posterior cingulate , retrosplenial , temporal/entorhinal	
	RM-11	V, VI	P, M	r/m	PT, CM	superficial $\geq$ deep	anterior/posterior cingulate , temporal/entorhinal	
	RM-17	V, VI	P, M	m	PT	superficial $\geq$ deep	anterior/posterior cingulate , retrosplenial , temporal/entorhinal	
	RM-18	V, VI	dense + "holes"	r(m)	PT	all layers	anterior/posterior cingulate , temporal/entorhinal	
	RM-8	V, VI	P (M)	I	PT, VM, Rh	deep fibres	anterior/posterior cingulate , temporal/entorhinal , retrosplenial	
(iv) PRELIMBIC/ MEDIAL ORBITAL	RM-29	V, VI	P, M	I	VM, PT	superficial > deep	anterior/posterior cingulate	corpus callosum , nucleus accumbens , zona incerta

(v) ANTERIOR CINGULATE	CSI-5	V	M	-	AM, AD, AV	superficial >>> deep	-	corpus callosum, nucleus accumbens, internal capsule, zona incerta, central gray area, medial lemniscus
	RM-23	V, VI	P, M	(light pl)	AM, AD, PC	superficial >>> deep	retrosplenial	
	RM-30	V, VI	dense + "holes"	(pl)	VM, CM, CL	superficial >>> deep	anterior/posterior cingulate, retrosplenial	
	CSI-21	V, VI	M	-	CM, VL, VM	all layers (labelling poor)	FrPaSS	corpus callosum, zona incerta, claustrum, internal capsule, entopeduncular nucleus
(vi) MEDIAL AGRANULAR	RM-13	V	M	-	VL	deep	retrosplenial	
	RM-20	V	M	-	VL	all layers (labelling poor)	medial agranular/FrPaM	
	RM-21	V, VI	P, M	I	CM	deep ≥ superficial	temporal/entorhinal, FrPaSS	
	CSI-1	V	P, M	I	CM, CL, AM	superficial = deep	anterior/posterior cingulate, retrosplenial	
(vii) PRELIMBIC/ ANTERIOR CINGULATE	CSI-9	V, VI	P	pl=I	VM, CM	deep >>> superficial	anterior/posterior cingulate	corpus callosum, nucleus accumbens, claustrum/dorsal endopiriform nucleus, internal capsule, zona incerta, central gray area, medial lemniscus, central amygdaloid nucleus
	CSI-10	V, VI	P	I	CM, CL	deep >>> superficial	anterior/posterior cingulate, medial agranular/FrPaM	
	CSI-11	V	P	l=pl	CM	deep >>> superficial	anterior/posterior cingulate	
	RM-16	V, VI	P, M	r/m (m=I)	CM (PT)	superficial > deep	-	
	RM-19	V	dense + "holes"	-	CM, VL, CL	superficial > deep	anterior/posterior cingulate	
	RM-22	superficial V, III	dense + "holes"	I (light pl)	CL	all layers	anterior/posterior cingulate, retrosplenial, medial agranular/FrPaM	
	RM-28	V, VI	P, M	I	CM, PT	superficial ≥ deep	-	
	RM-31	V, VI	P, M	I	CM, VM	deep	-	
(viii) PRELIMBIC/ ANTERIOR CINGULATE/ INFRALIMBIC	CSI-8	deep III, V, VI	P, M	pl=I	PT, VM, PC, CM	superficial >> deep	anterior/posterior cingulate, retrosplenial	nucleus accumbens, corpus callosum, zona incerta, central gray area

- (b) predominant labelling of matrix, as shown by comparing sections with adjacent CaBP stained sections, in the dorso-lateral/lateral regions.
- (c) Inputs to both patch and matrix, which could be further divided into two distinct groups:
  - (i) those which displayed light labelling of both patch and matrix, usually situated in ventromedial and medial areas of the striatum
  - (ii) dense labelling of matrix and some patches within the central area of striatum, with other patches within the same small area receiving little or no cortical input. This gave the appearance of there being small "holes" in the labelling akin to those described by Donoghue and Herkenham (1986). This labelling pattern tended to be fairly widespread in the dorsoventral axis but mainly in rostral and medial areas.

#### Analysis of PhAL labelling

Each group of cases was examined closely to determine if any correlation existed between the type of striatal labelling present and that observed in other areas of the brain. In addition each group was compared to other groups to highlight the differences between labelling patterns due to the use of different injection sites.

The results are summarised in Table 3.

FIGURES 9, 11, 13, 15, 17, 19, 21, 23:

Camera lucida drawings of injection sites, contralateral cortex labelling, and thalamic labelling of specific cases.

- A. Position of PhAL injection within the cortex. (Distance from Bregma is approximate).
- B. Enlarged view of injection site showing labelled perikarya, and corresponding area in contralateral cortex showing labelled terminals and fibres.
- C. 1-4 Locations of PhAL labelling in thalamic nuclei (anterior-posterior), with particular reference to the medio dorsal thalamic nucleus and its subdivisions.

Abbreviations:

AD	anterodorsal thalamic nucleus	MDM	mediodorsal thalamic nucleus, medial portion
AM	anteromedial thalamic nucleus	MDpl	mediodorsal thalamic
AV	anteroventral thalamic nucleus	PC	paracentral thalamic nucleus
CL	centrolateral thalamic nucleus		
CM	centromedian thalamic nucleus	(pl)	paralamellar portion of MD nucleus
DG	dentate gyrus	PO	posterior thalamic nuclear group
fmi	forceps minor	PT	paraterial thalamic nucleus
fr	fasciculus retroflexus	PVA	paraventricular thalamic nucleus anterior part
Hbl	lateral habenular nucleus	PVP	paraventricular thalamic nucleus posterior part
Hbm	medial habenular nucleus	Re	reuniens thalamic nucleus
IMD	intermediodorsal thalamic nucleus	Rh	rhomboid thalamic nucleus
LP	lateroposterior thalamic nucleus	Rt	reticular thalamic nucleus
MD	mediodorsal thalamic nucleus	Sm	nucleus of stria medullaris
MDC	mediodorsal thalamic nucleus, central portion	VL	ventrolateral thalamic nucleus
		VM	ventromedial thalamic nucleus



FIGURES 10, 12, 14, 16, 18, 20, 22, 24:

Camera lucida drawings of various striatal sections from specific cases showing the location of PhAL labelling.

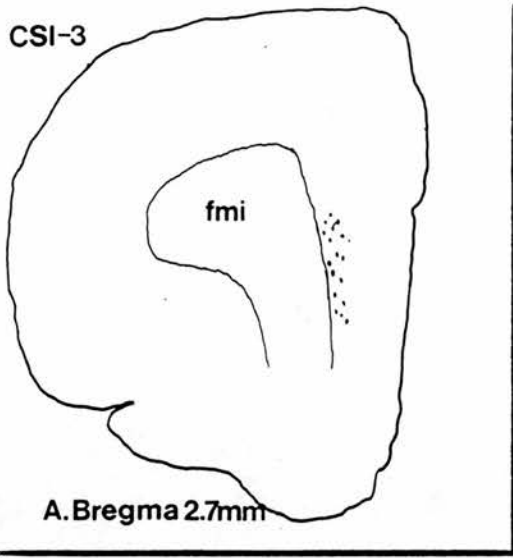
Positions relative to Bregma are approximate.

Abbreviations:

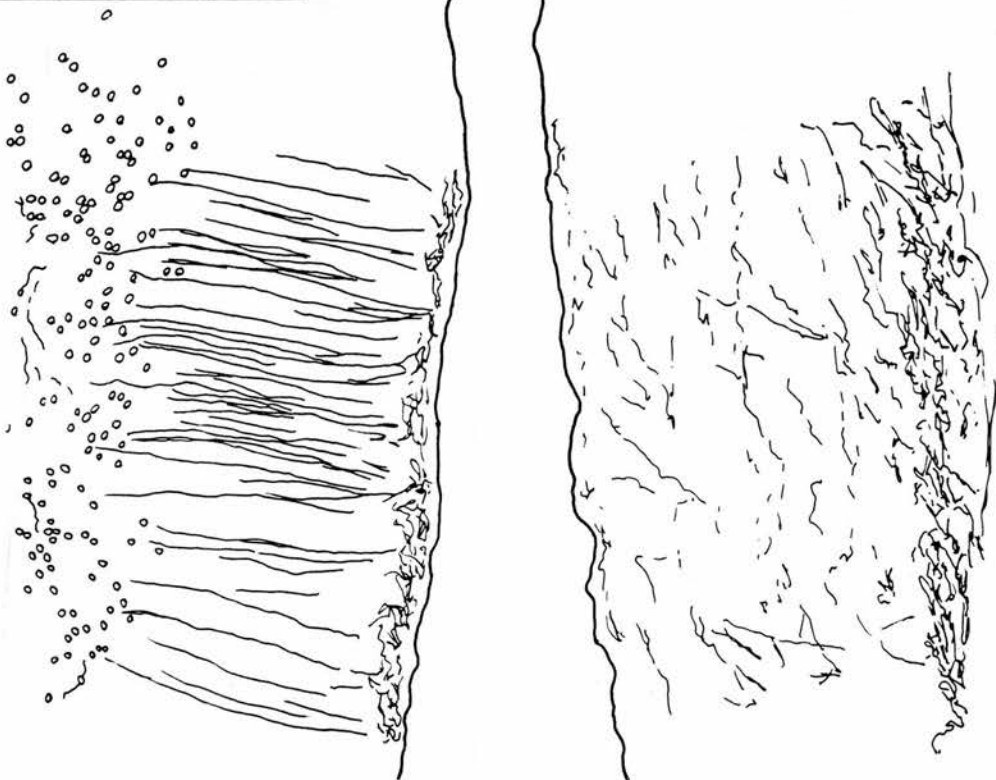
ac	anterior commissure	FB	fibre bundles
BSTL	bed nucleus of stria terminals, lateral portion	fmi	forceps minor
cc	corpus callosum	FStr	fundus striati
cg	cingulum	gp	globus pallidus
Cl	claustrum	ic	internal capsule
DEN	dorsal endopiriform nucleus	Pv	lateral ventricle
ec	external capsule	Na	nucleus accumbens

CSI-3

FIGURE 9



B



C

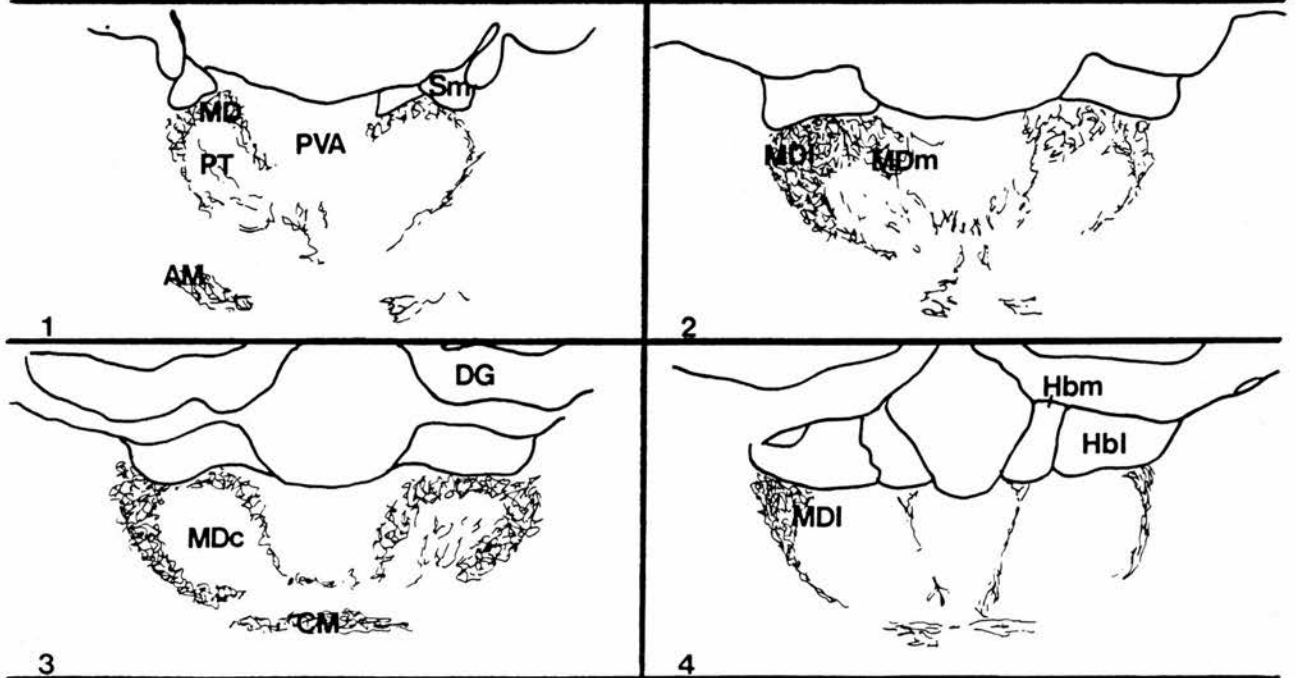
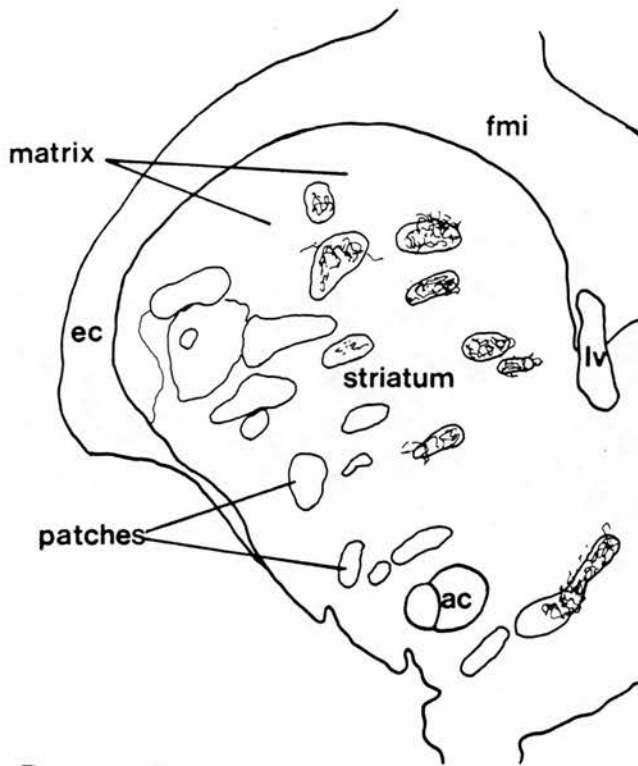


FIGURE 10

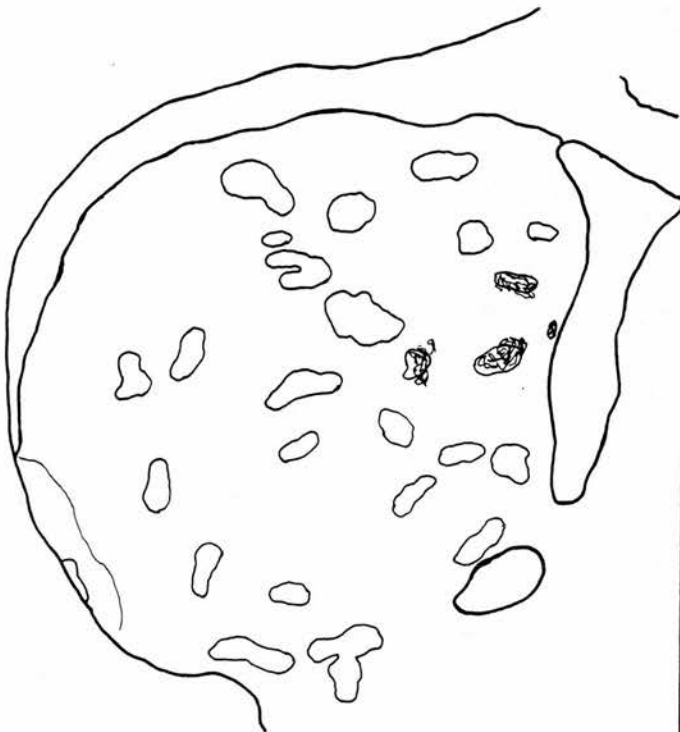
CSI-3



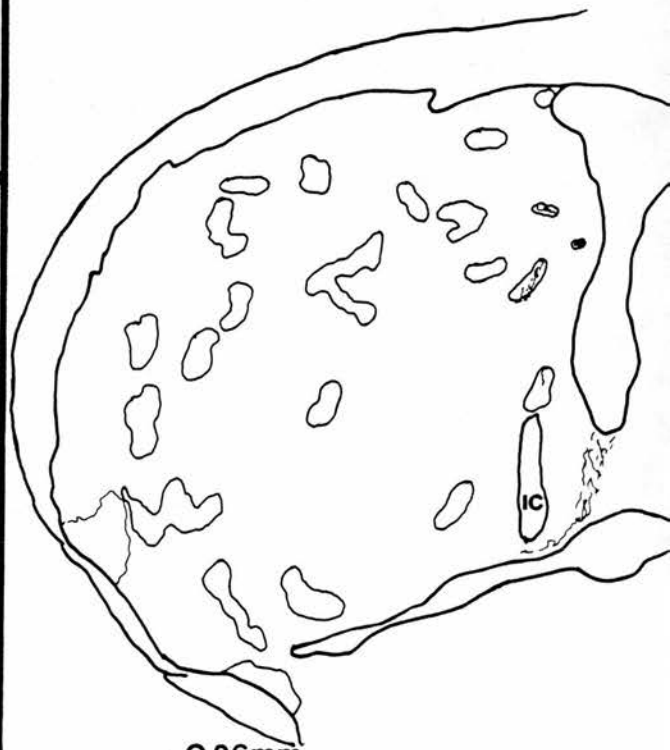
Bregma 1.7mm



1.6mm



0.48mm



-0.26mm

(i) Prelimbic cortex only

Out of a total of three cases which received injections principally into this cortical region, two displayed terminal labelling in patches within the striatum (see Figure 10). Examination of the thalamic nuclei revealed that these cases had dense labelling within lateral (l) and paralamellar (pl) regions of the mediodorsal nucleus (MD) (Figure 9C) with slightly less labelling in centromedian (CM), ventrolateral (VL) and paratenial (PT) thalamic nuclei. The contralateral area of cortex corresponding to the injection site in both cases displayed some evidence of inputs from the ipsilateral cortex, in that all cortical layers had terminal labelling with deeper layers displaying more intrinsic labelling (Figure 9B). Cortical inputs were similar for both cases; there was some evidence of projections to anterior/posterior cingulate cortex, while only one case also displayed labelling in retrosplenial cortex.

The injections site in the remaining case clearly projected to both patch and matrix within the striatum while most thalamic labelling was in MDl, CM and centrolateral nuclei (CL). Only deep fibres and superficial terminals were labelled in homologous cortex, and no other cortical areas showed any evidence of terminal labelling.

Examination of other brain areas in all three cases revealed the presence of dense fibre labelling within the corpus callosum, as well as dense terminal labelling in the nucleus accumbens and to a lesser extent within the claustrum.

FIGURE 11

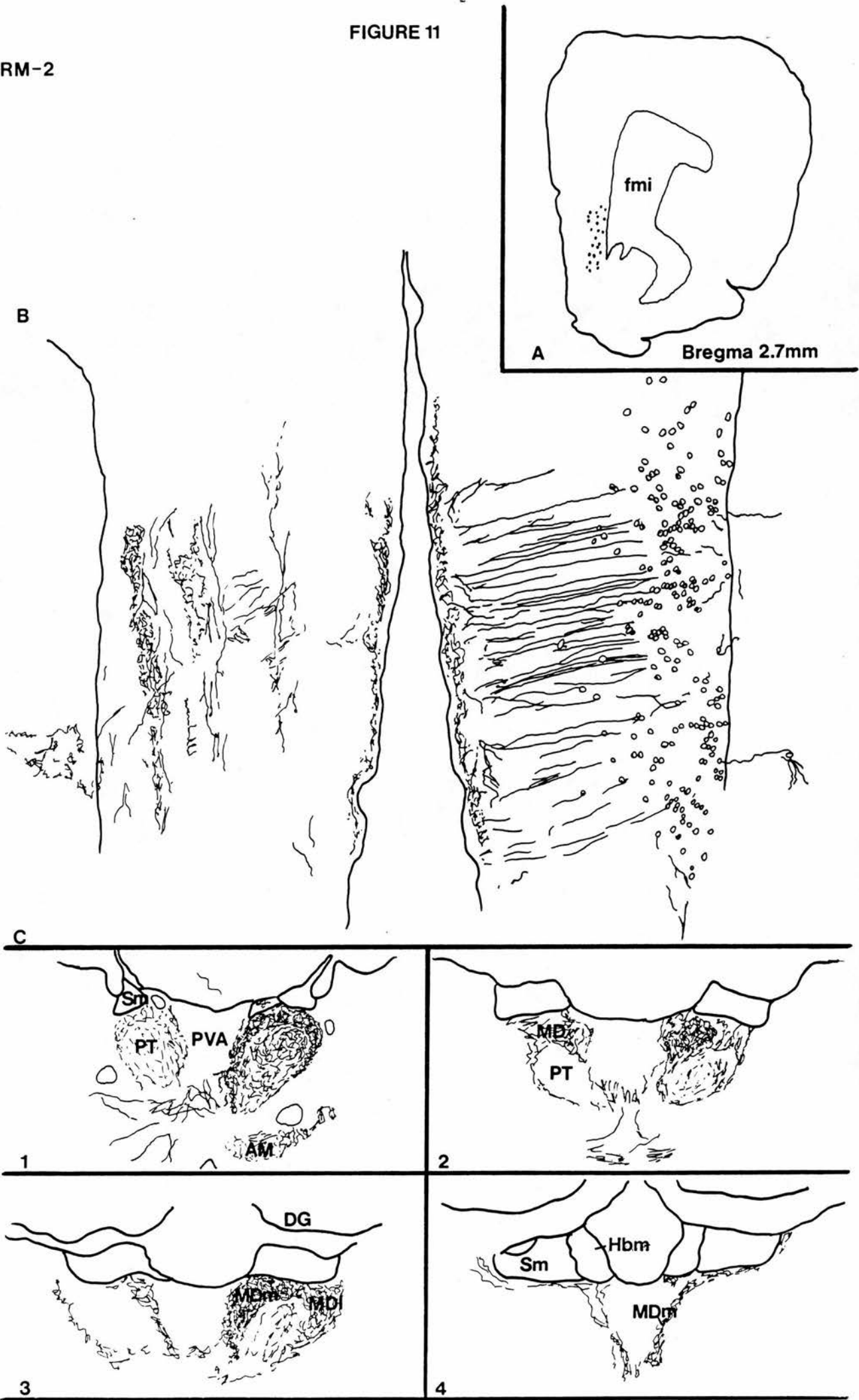
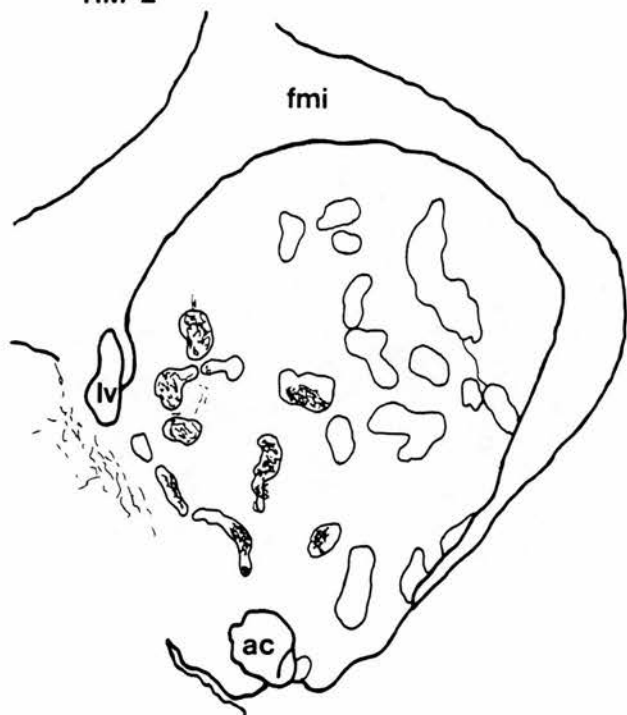
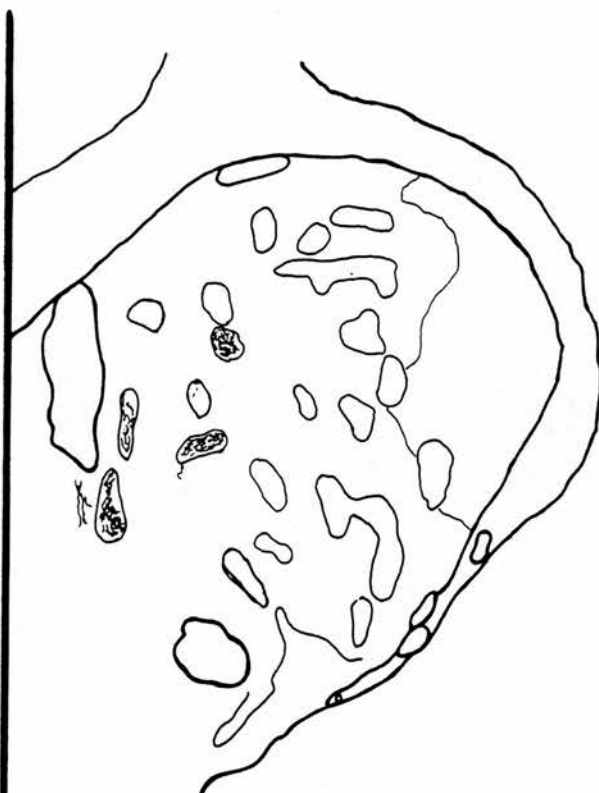


FIGURE 12

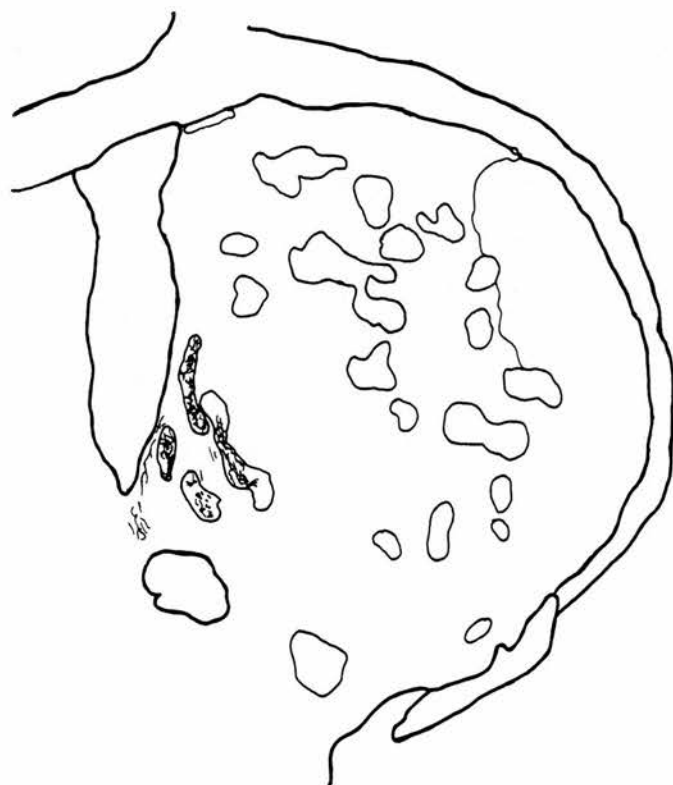
RM-2



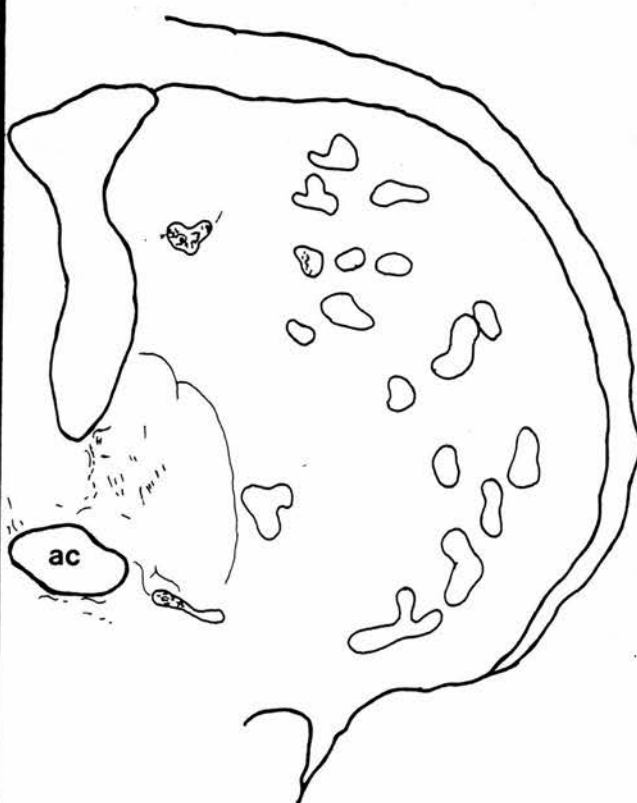
Bregma 1.7mm



1.6mm



0.7mm



0.2mm

(ii) Infralimbic cortex only

Three cases appeared to have perikarya labelled only in the infralimbic cortex, and also displayed dense labelling within the striatum which corresponded to patches. Cases RM-2 (shown in Figures 11 and 12) and RM-7 also displayed inputs to the medial portion of the MD (MDm) and PT thalamic nuclei and mainly deep layers of the homologous cortex. The remaining case (RM-6) however, had dense inputs to the rostral-most part of the MD nucleus (MDr) with labelling to a lesser extent in MDl and MDm, more caudally. In addition, this case had only labelled fibres in the deep layers of the contralateral cortex homologous to the injection site. Both RM-2 and RM-6 had some light projections to anterior/posterior cingulate cortex while only RM-7 showed labelling in temporal/entorhinal cortex.

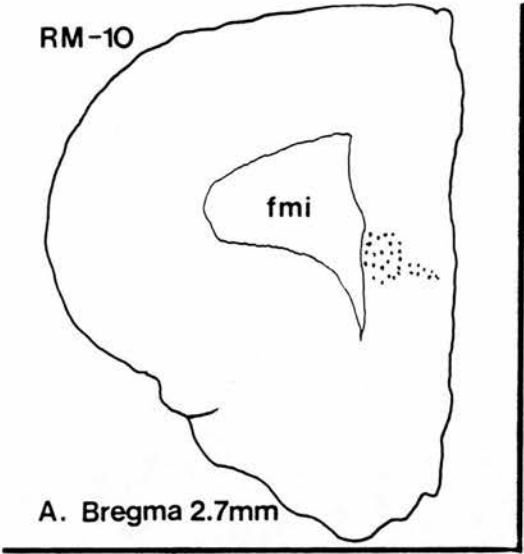
Other brain areas displaying PhAL labelling were corpus callosum, nucleus accumbens, claustrum, central gray area and bed nucleus of the anterior commissure.

(iii) Injection into both prelimbic and infralimbic cortex

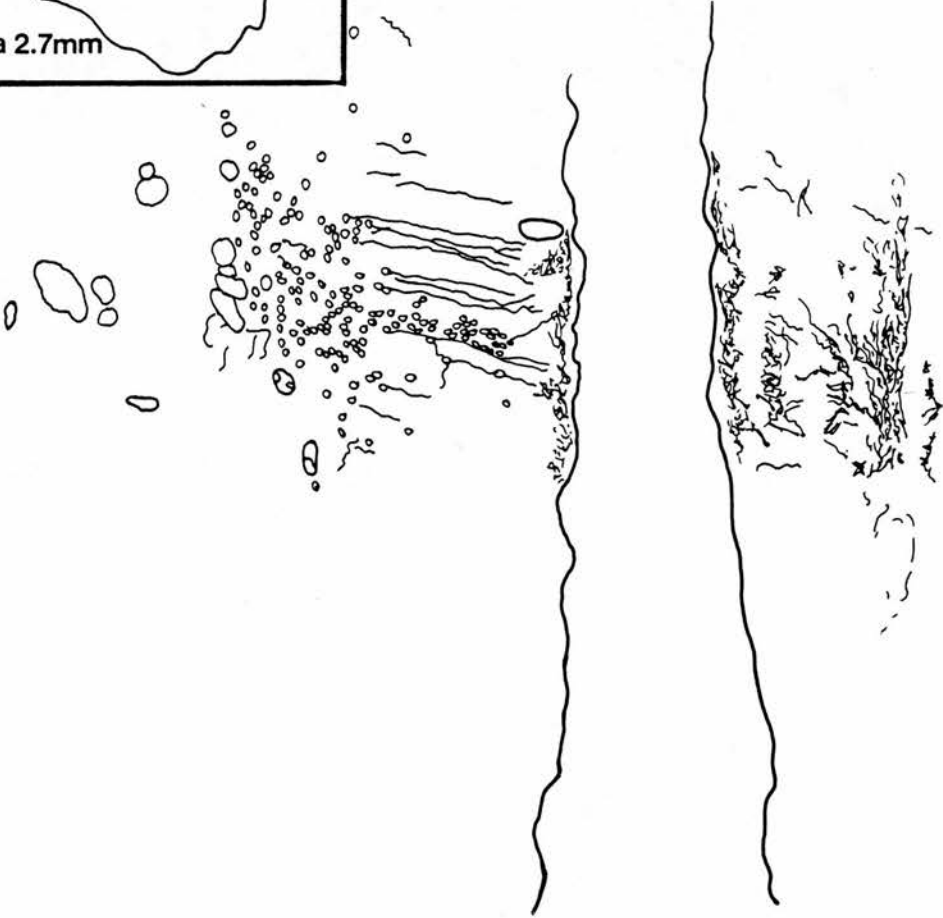
With the cases in this group it was obvious that cells in both areas of cortex had taken up PhAL as this was reflected in the projection patterns observed for each case.

All injections in this group labelled both patches and matrix within the striatum. Four cases displayed labelling as described for type (c)(i) and three of these also showed dense labelling in the MDr/MDm. The fourth case, RM-3 projected mainly to MDl but this was probably due to the fact that some medial orbital neurons had taken up the tracer. All of these cases, apart from RM-10

FIGURE 13



B



C

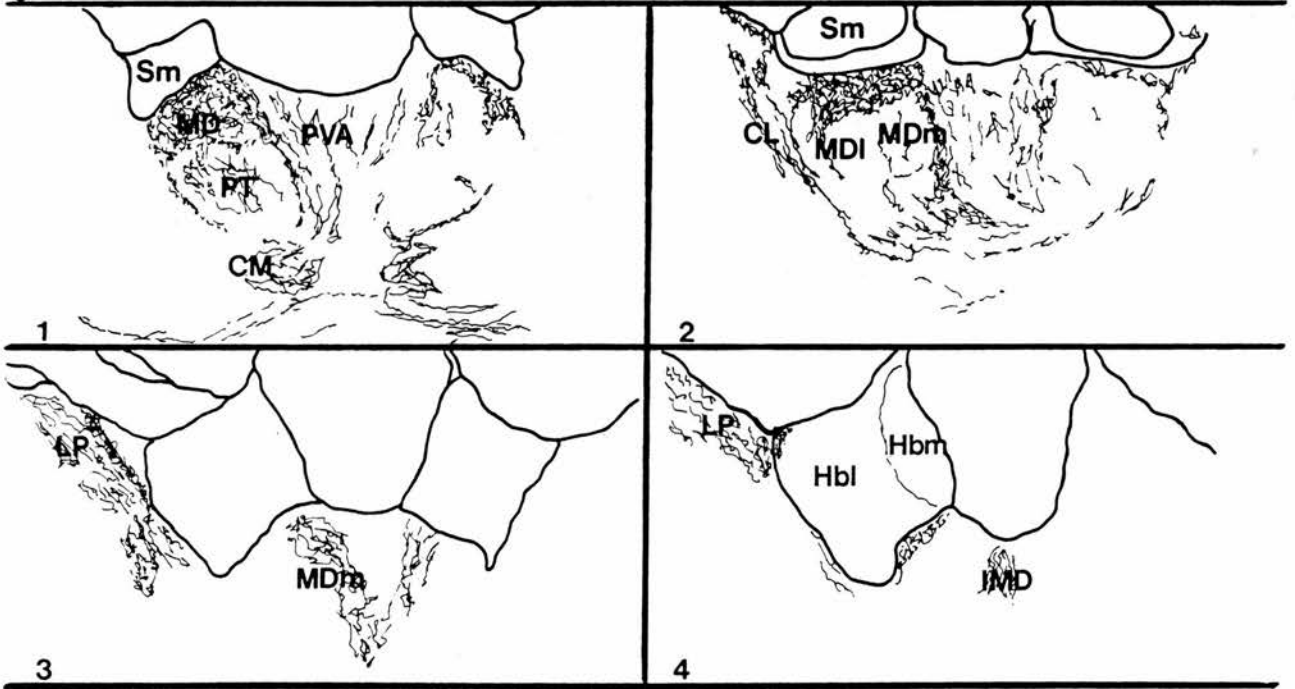
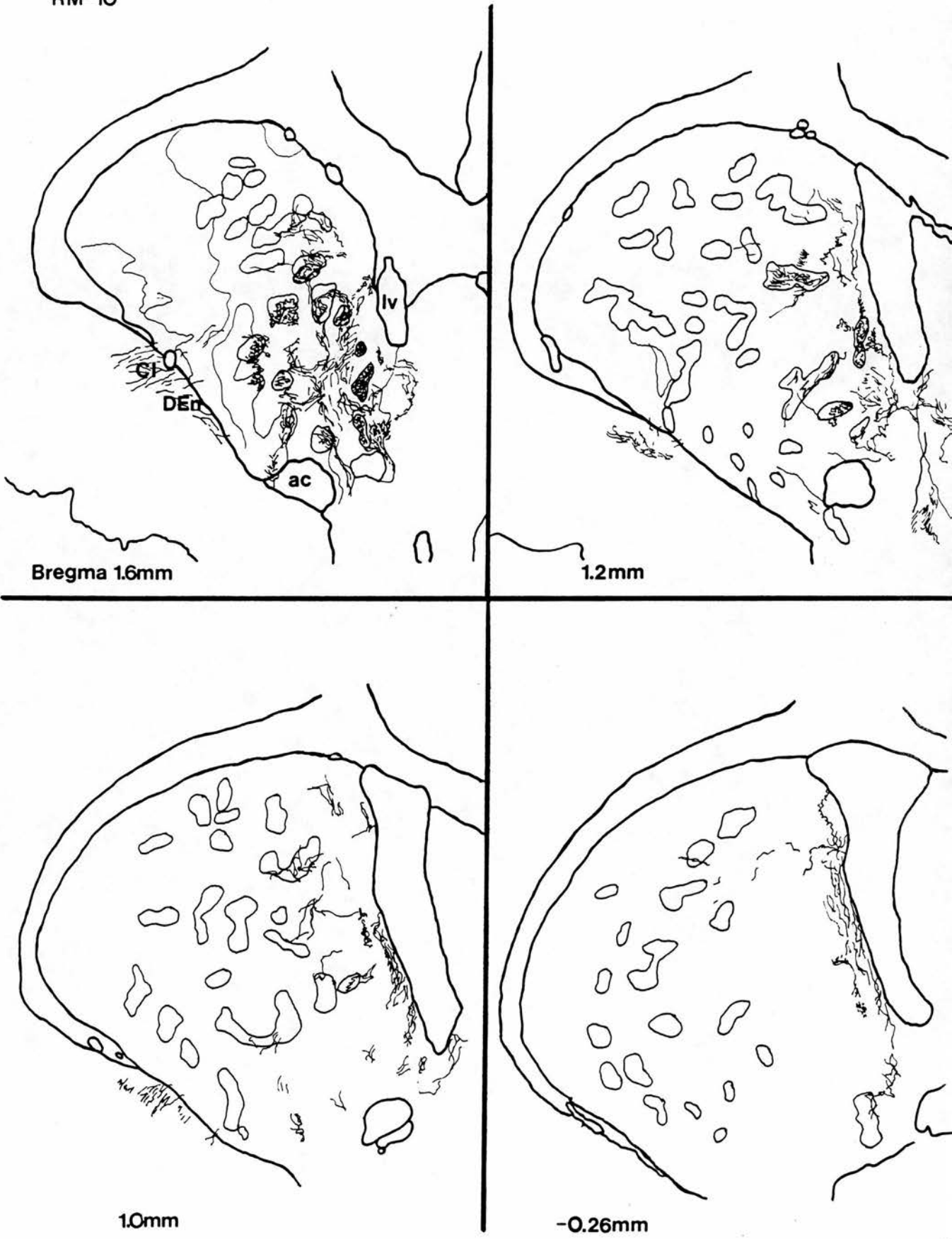




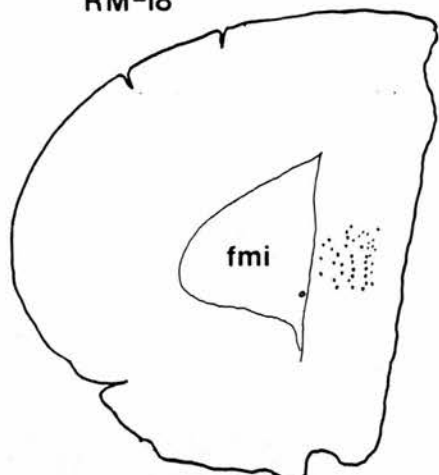
FIGURE 14

RM-10



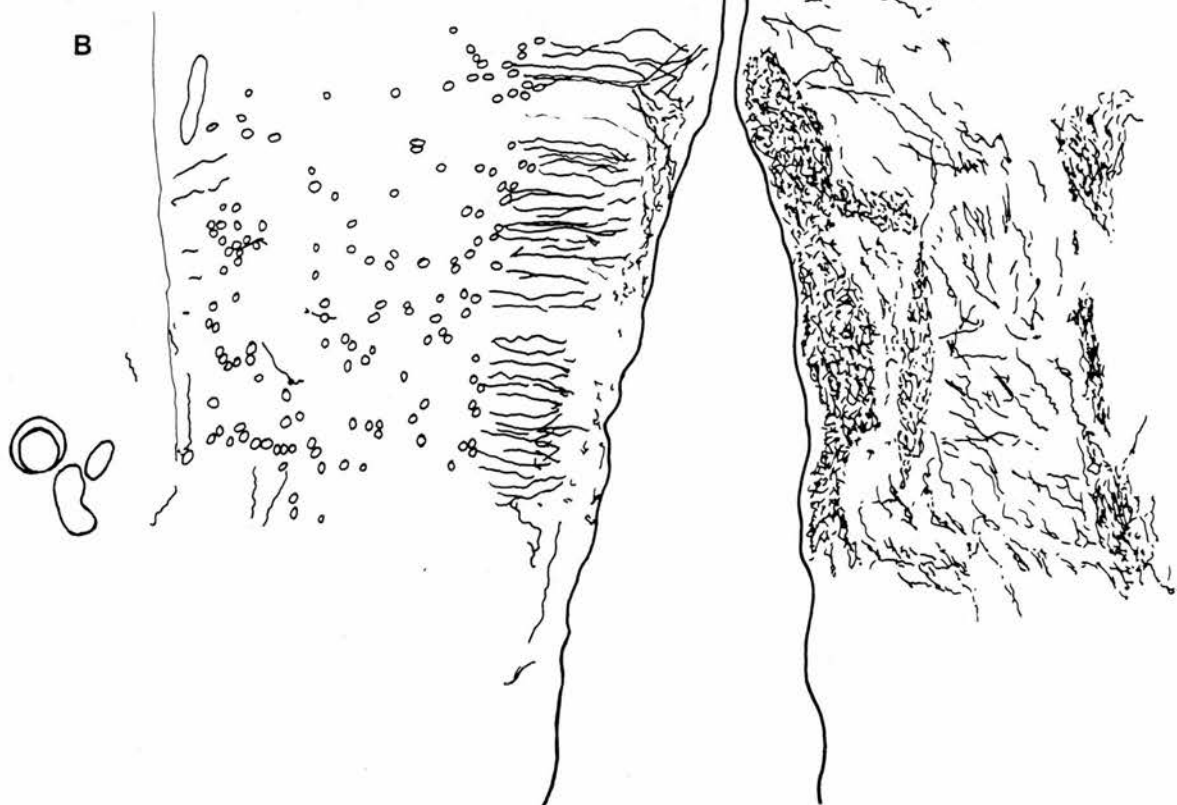
RM-18

FIGURE 15

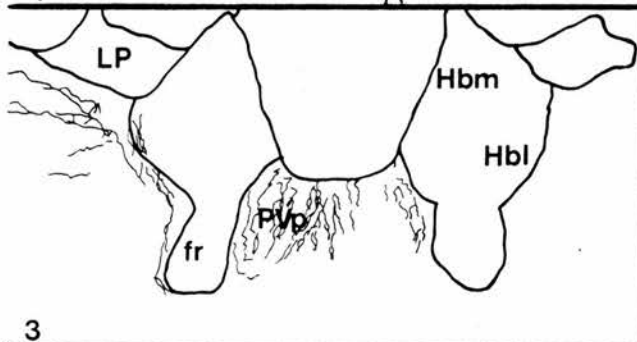
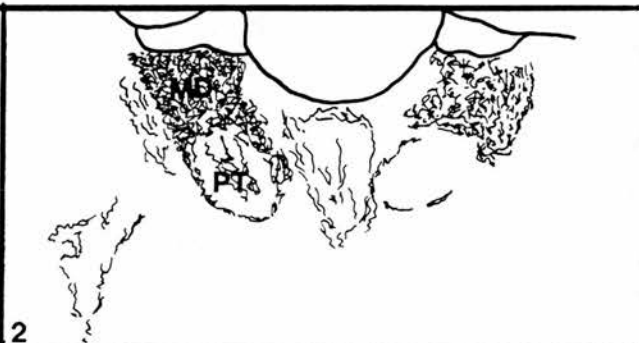
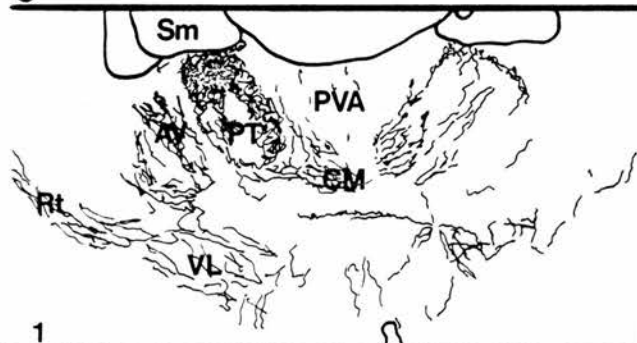


A. Bregma 3.2mm

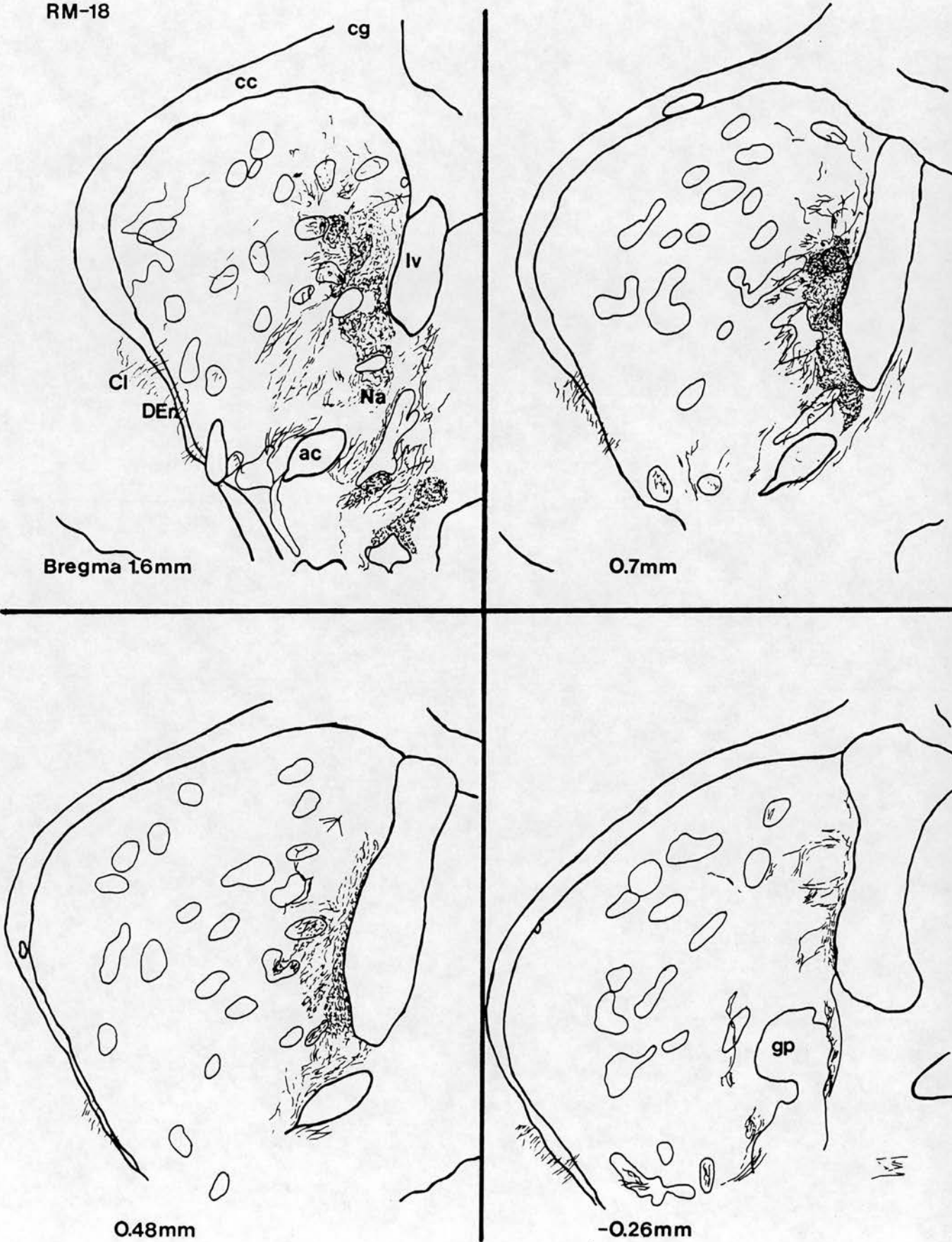
B



C



RM-18



(shown in Figures 13 and 14) also had projections to the PT thalamic nucleus, while RM-10 and RM-11 also displayed projections to CM nucleus.

Examination of the contralateral homologous cortex revealed that all cases displayed some labelling throughout all cortical layers, although the most dense labelling tended to be in superficial layers. Other cortical areas which had the most labelling were anterior/posterior cingulate and temporal/entorhinal cortex, with some labelling present in retrosplenial cortex.

Other brain areas displaying most PhAL labelling were corpus callosum, nucleus accumbens, internal capsule, central gray area, zona incerta, globus pallidus and claustrum.

The striatal labelling present in the two remaining cases RM-9 and RM-18 (shown in Figures 15 and 16) was slightly different to the rest of the cases in this section in that although both patch and matrix were labelled, it was only some patches which received dense inputs while others within the same small area received little or no cortical input (type (c)(i)).

Both cases also had dense projections to rostral/medial MD nucleus and PT thalamic nucleus, all layers of the contralateral homologous cortex, and anterior/posterior cingulate and temporal/entorhinal cortices.

Other brain areas labelled were similar to those labelled in the rest of the cases in this section.

#### (iv) Injections into prelimbic and medial orbital areas

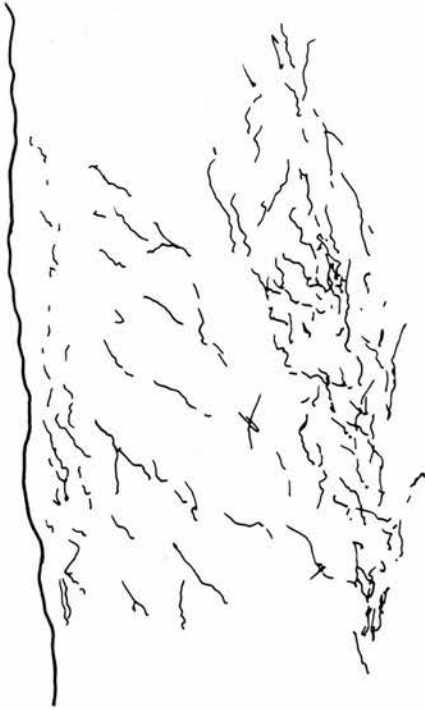
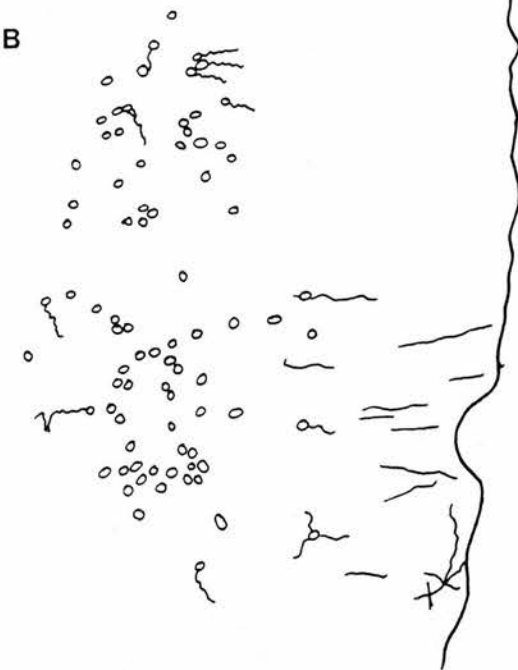
Two cases (RM-8 and RM-29) were classified in this category although RM-3 (section (iii)) had some neurons labelled in the

RM-8

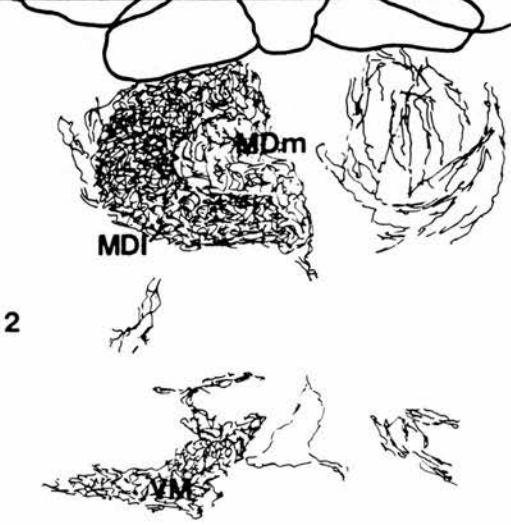
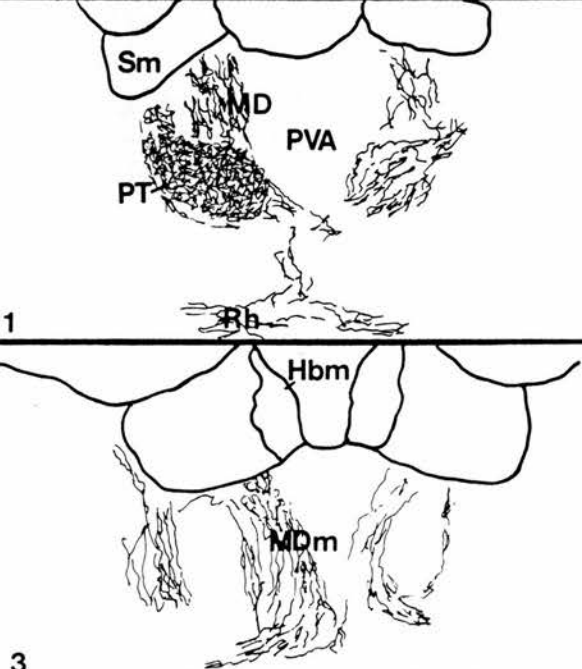
FIGURE 17

A. Bregma 4.2mm

B



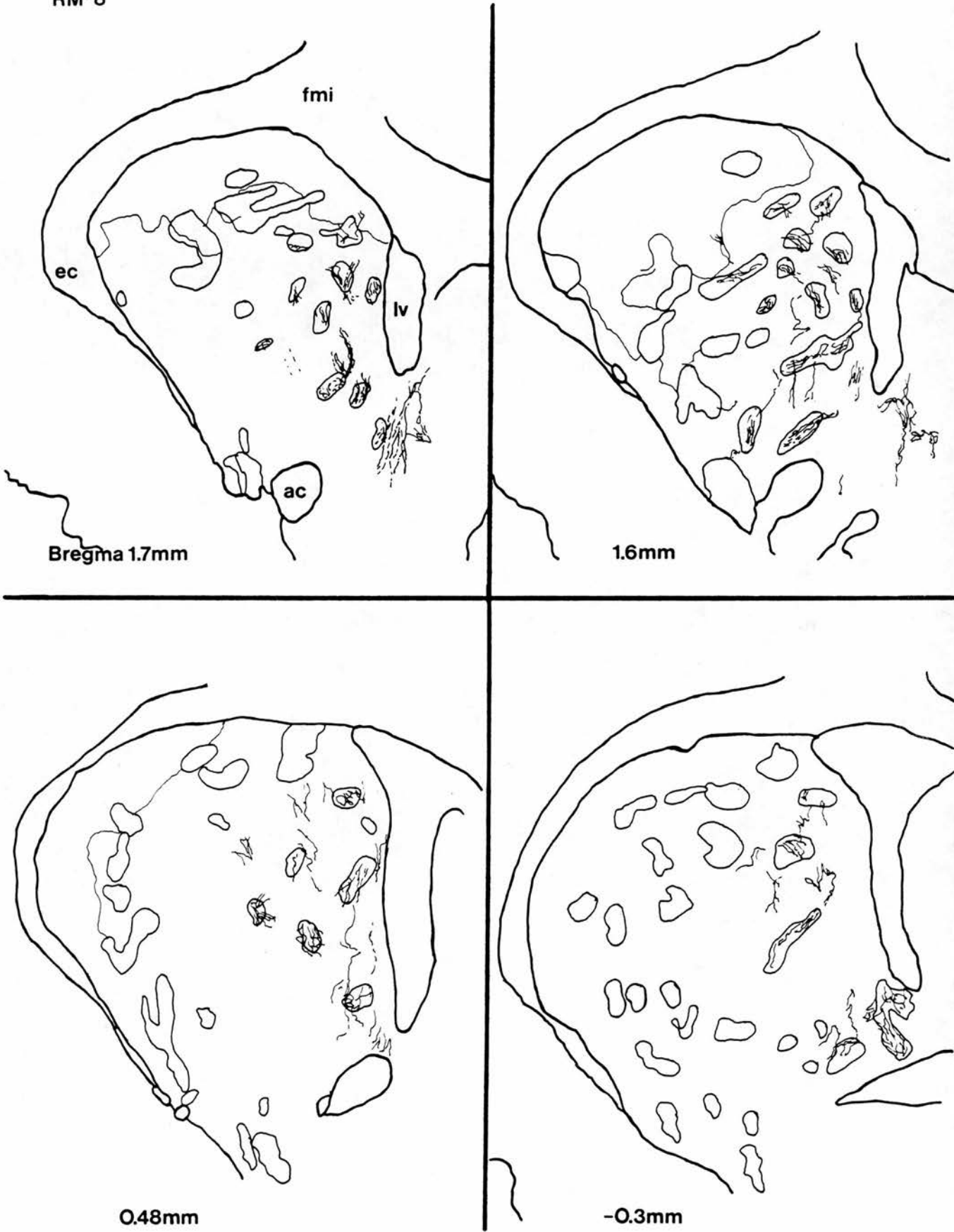
C



3

FIGURE 18

RM-8



medial orbital areas as well as prelimbic and infralimbic areas, and the bulk of injected neurons tended to be more caudal than those of RM-8 or RM-29.

Both RM-8 (see Figures 17 and 18) and RM-29 had the same pattern of thalamic labelling as RM-3 that is PT and MD1 thalamic nuclei with some dense labelling in the ventro medial (VM) nucleus, and similar cortical projections; both displayed inputs to anterior/posterior cingulate cortex, while other areas labelled were corpus callosum, nucleus accumbens and zona incerta.

Their patterns of labelling differed in the respect that RM-8 also displayed labelling in retrosplenial and entorhinal/temporal cortices, while the predominant striatal labelling was present in patches (and some in matrix) with labelling mainly in deeper layers of the homologous cortex. RM-29 on the other hand had projections to both striatal patch and matrix compartments with terminal labelling of a greater extent in superficial layers of the contralateral homologous cortex.

(v) Injections into anterior cingulate cortex only

Only three cases appeared to have injections principally within this cortical area, although RM-30 also showed evidence of medial agranular cortical neuron involvement. All three cases showed fairly similar labelling patterns. For CSI-5, (see Figures 19 and 20) only striatal matrix received projections from the labelled cortical neurons, while anterodorsal (AD), anteromedial (AM) and anteroventral (AV) thalamic nuclei received the most dense thalamic inputs. All layers of the contralateral homologous cortex were labelled although most labelling occurred superficially and there were no inputs to other cortical areas.



CSI-5

FIGURE 19

A Bregma 0.7mm

B

C

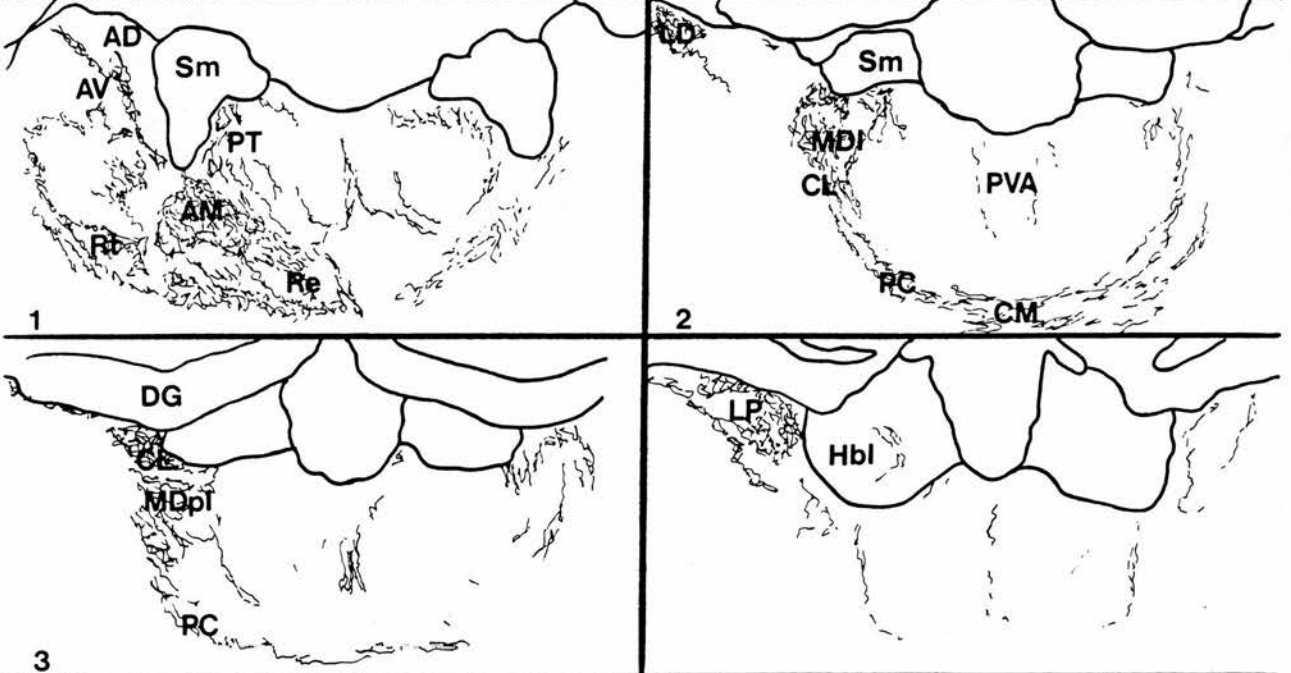
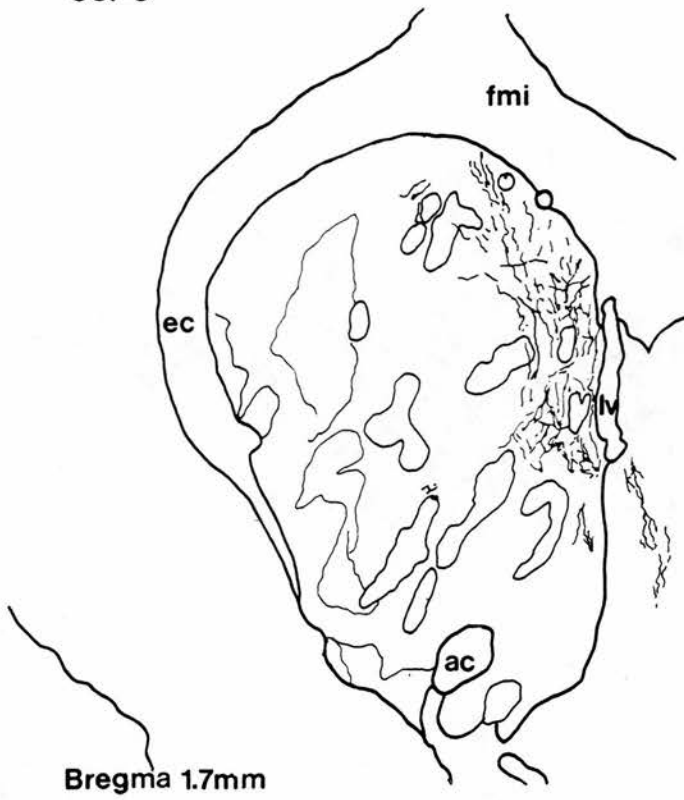




FIGURE 20

CSI-5



RM-23 on the other hand displayed inputs to both patch and matrix (although mainly matrix) but also had projections to AD, AM and also the paracentral (PC) thalamic nucleus, with labelling throughout the homologous cortex and very light MDpl labelling. The retrosplenial cortex in this case also displayed light PhAL labelling. In the case of RM-30 (with some medial agranular neurons labelled) the striatum displayed a distribution of labelled inputs similar to RM-9 and RM-18 (see earlier) in that dense labelling was present in the central area of matrix plus some patches with "holes" within the labelling which corresponded to patches lacking in CaBP-immunoreactivity. The major thalamic inputs arising from this injection were to CM, VM, CL and only light staining in MDpl, suggestive of medial agranular neuron involvement (see later). Superficial contralateral homologous cortical layers were stained densely with very slight labelling in other layers, and anterior/posterior cingulate and retrosplenial cortex also appeared to receive cortical inputs.

Other brain areas receiving inputs were similar for each case; corpus callosum, nucleus accumbens, internal capsule, zone incerta, central gray and medial lemniscus displayed most labelling.

#### (vi) Medial agranular cortex injections

Out of four cases with medial agranular injections, RM-13 (shown in Figure 21 and 22), RM-20 and CSI-21 displayed preferential labelling of striatal matrix only, while the fourth (RM-21) projected to both patch and matrix.

The major thalamic input of the three 'matrix only' cases (i.e. RM-13, RM-20, CSI-21) was the VL nucleus, with no labelling whatsoever in the MD thalamic nucleus. CSI-21 also projected to CM,

FIGURE 21

RM-13

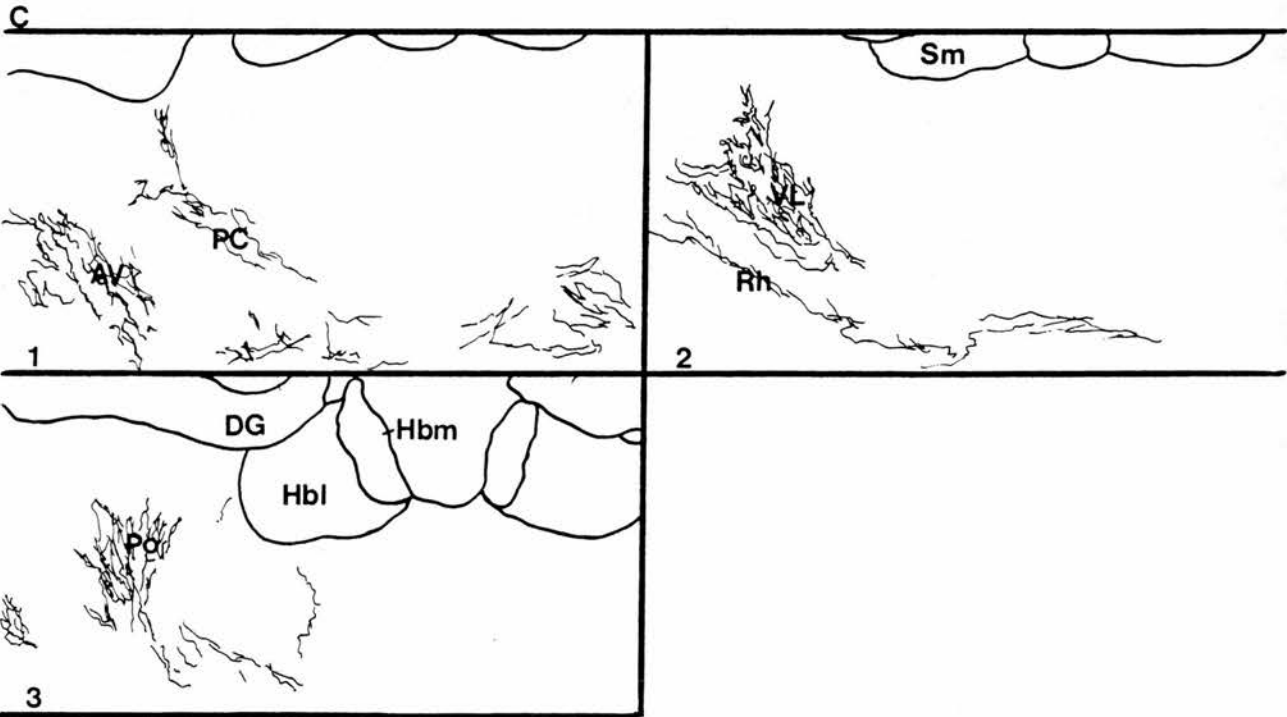
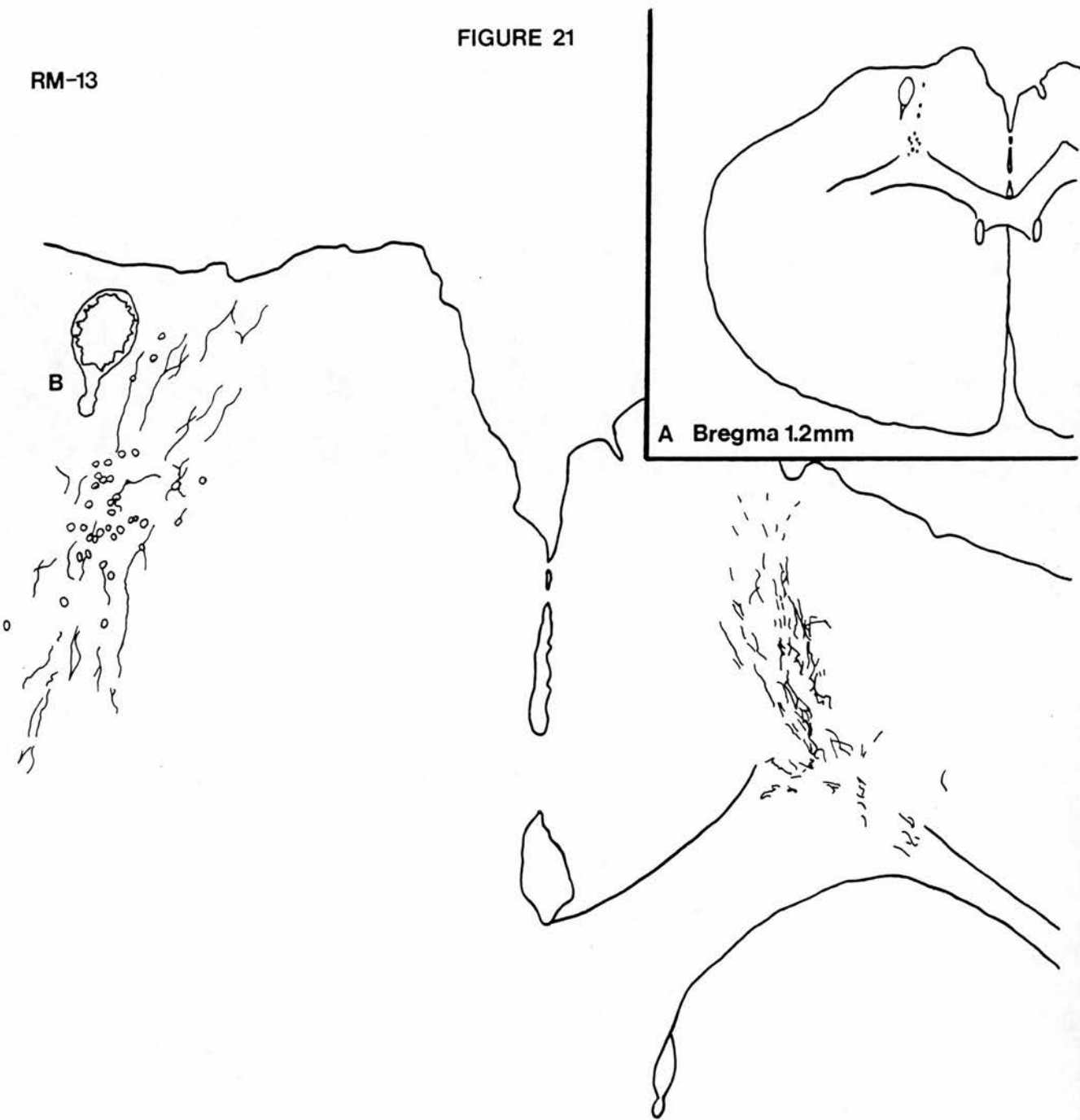
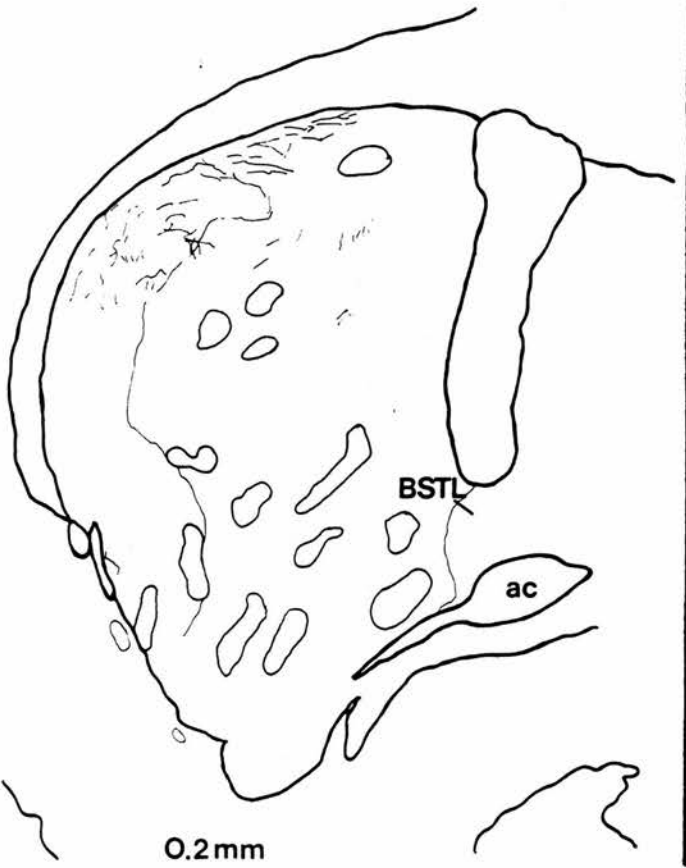
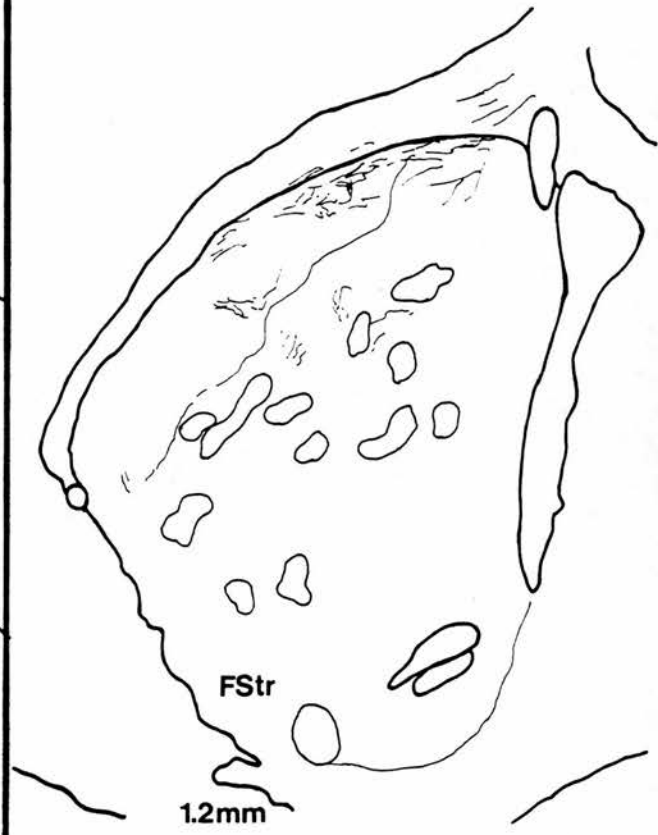
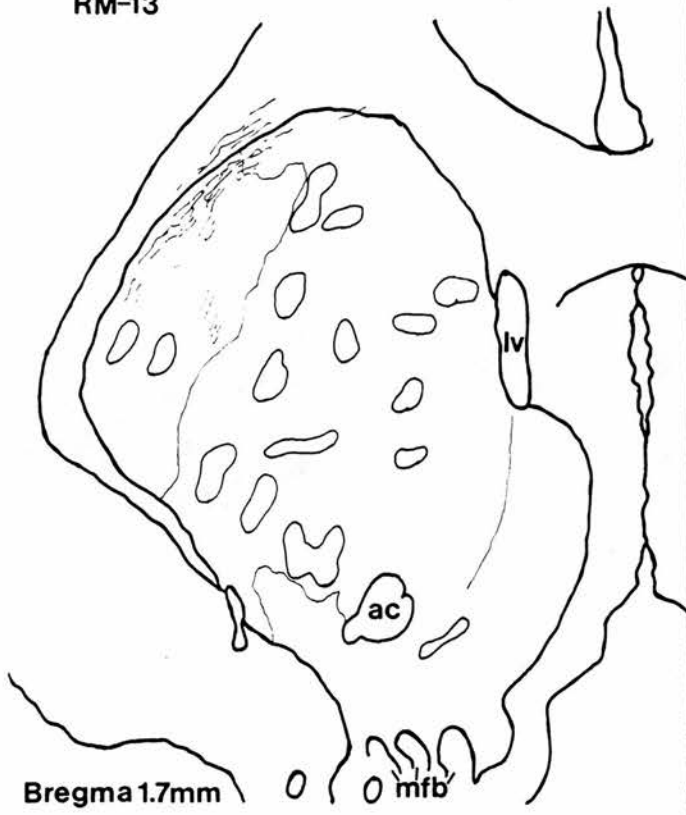


FIGURE 22

RM-13



as did RM-21. Contralateral homologous cortical labelling was poor, as was labelling in other cortical areas. However, those cases which had reasonable labelling indicated that homologous cortex received inputs to either all layers or deep fibres. Cortical labelling tended to be present mainly in motor areas and also retrosplenial and temporal/entorhinal cortex.

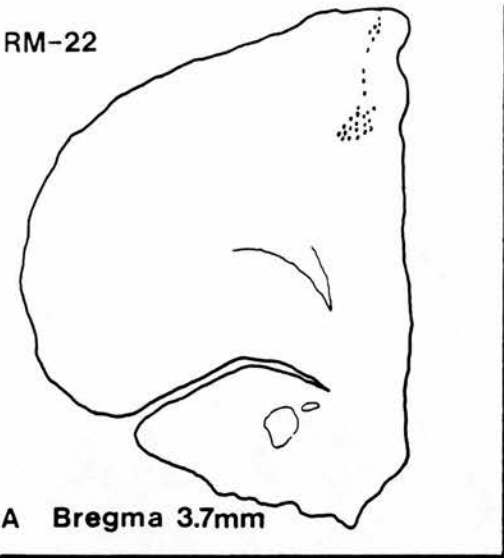
Corpus callosum, zona incerta, claustrum, internal capsule and entopenduncular nucleus were the other brain areas which displayed some PhAL labelling.

(vii) Prelimbic/anterior cingulate cortex injections

All of the cases in this group demonstrated perikaryal labelling in both cortical areas to a greater or lesser degree, which tended to be reflected in the distribution of labelling observed.

Out of nine cases, three had preferential labelling in striatal patches, and six had projections to both patch and matrix, two of which displayed type (c)(ii) labelling (RM-19, RM-22).

All cases with projections to patches (CSI-9, CSI-10, CSI-11) showed very similar labelling patterns to those cases in group (i) (prelimbic cortex injections only) which projected preferentially to patches. The presence of dense labelling in lateral/paralamellar regions of MD nucleus and CM (VM for case CSI-9) was indicative of a prelimbic injection. Like similar prelimbic cortex only injections, all projected to all layers of the contralateral homologous cortex with the most dense labelling in deepest layers, and all displayed labelling in anterior/posterior cingulate cortex (CSI-10 also had light projections to agranular cortex). Thus it would appear that even though some anterior cingulate neurons took up PhAL only the



A Bregma 3.7mm

B



C

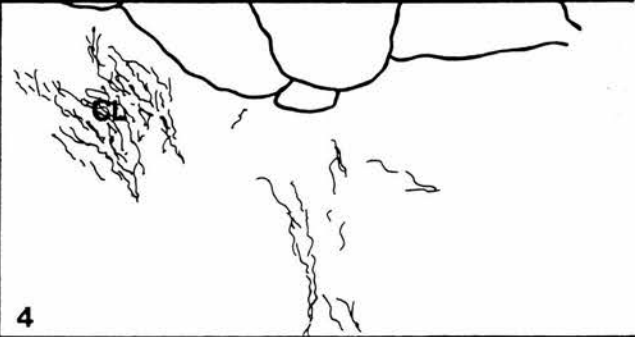
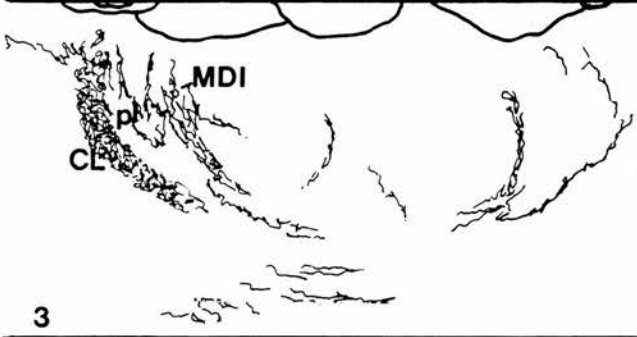
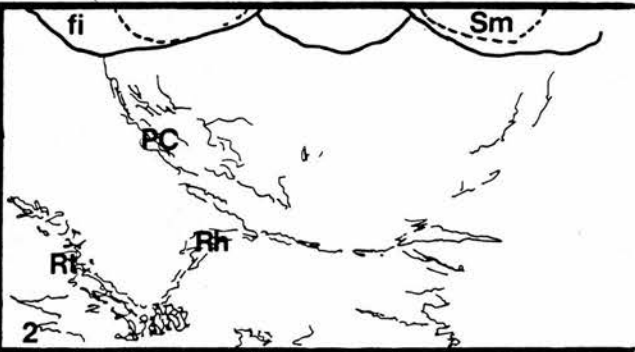


FIGURE 24

RM-22



Bregma 1.7mm



1.6mm



1.0mm



0.2mm

labelling pattern associated with strictly prelimbic injections was observed in these cases.

Cases CSI-1, RM-16, RM-28, RM-31 displayed projections to both patch and matrix and all projected to CM thalamic nucleus. However, there was some variation in their MD nucleus labelling: CSI-1, RM-28 and RM-31 projected preferentially to MDl while RM-16 had inputs mainly to rostral MD with less labelling in both MDl and MDm. RM-16 and RM-28 also had well labelled PT thalamic nuclei, perhaps suggesting the involvement of some infralimbic neurons in the projection patterns of these cases. Similarly, RM-31 appeared to have some medial agranular neuron involvement by virtue of the dense labelling observed in VM and VL thalamic nuclei.

In terms of homologous cortical labelling, two out of the four cases had dense superficial terminal labelling while the other two CSI-1 and RM-31 labelled all layers and only deep fibres respectively. Only case CSI-1 displayed any other cortical labelling and this was in the anterior/posterior cingulate cortex and retrosplenial cortex.

RM-22 (shown in Figures 23 and 24) and RM-19 which displayed type (c)(ii) labelling (dense labelling plus "holes") both had dense labelling in CL thalamic nuclei, all layers of the contralateral homologous cortex and anterior/posterior cingulate cortex. Some medial agranular neurons may also have been injected in both cases as suggested by the patterns of thalamic labelling observed. In addition, motor cortex of RM-22 displayed some evidence of inputs, this again being a characteristic of medial agranular injections.

Other labelled areas were mainly corpus callosum, nucleus accumbens, claustrum/dorsal endopiriform nucleus, internal capsule,



zona incerta, central gray area, medial lemniscus and central amygdaloid nucleus.

(viii) Mixed prelimbic, anterior cingulate and infralimbic cortex injection

Only one case, CSI-8 had a very large injection site in which prelimbic, infralimbic and anterior cingulate neurons were labelled with PhAL. This was reflected in the labelling pattern observed. Both striatal patch and matrix were labelled, while the paralamellar and lateral regions of the MD nucleus received inputs of almost equal density.

Other thalamic inputs were principally to PT, VM, CM and PC nuclei. Superficial contralateral homologous cortical layers were labelled to a greater extent than other deeper layers, while most other cortical labelling was observed in anterior/posterior cingulate and retrosplenial cortex.

Nucleus accumbens, corpus callosum, zona incerta and central gray areas were the only other brain regions to display PhAL labelling.

## DISCUSSION

The present results confirm earlier findings of a topographical arrangement of corticostriatal inputs such that each cortical area provides inputs, not only to proximal striatal areas, but also more distal areas (Goldman and Nauta, 1977; Yeterian and Van Hoesen, 1978; Van Hoesen et al., 1981; Selemon and Goldman-Rakic, 1985). Similarly, further evidence is presented to show that corticostriatal terminals are distributed in distinct compartments, patch and matrix (Goldman, 1982; Goldman and Nauta, 1977; Jones et al., 1977; Kunzle, 1975; Royce, 1978), which coincide with neurochemically distinct compartments intrinsic to the striatum (Gerfen, 1984; Ragsdale and Graybiel, 1981, 1984; Donoghue and Herkenham, 1983, 1986; Graybiel, Ragsdale and Moon Edley, 1979). Furthermore, a correlation has been demonstrated between the area of cortex injected with PhAL and the striatal, thalamic, cortical and homologous contralateral cortical labelling.

### Thalamic projections

It was found that each particular cortical area injected with PhAL gave rise to a characteristic pattern of thalamic labelling. For example, all cases with prelimbic injections displayed dense labelling predominantly in MD1 and to a slightly lesser extent in MDm and CM thalamic nuclei. The pattern of thalamic labelling arising from several of the PhAL injected cortical areas tended to agree with those observed in previous studies (Beckstead, 1979; Krettek and Price, 1977; Leonard, 1969; Cornwall and Phillipson, 1988). These studies however did not closely examine the labelling

within regions of the MD nucleus to any great extent.

A much more definitive study was recently carried out by Groenewegen (1988). He injected HRP or WGA-HRP into different parts of MD thalamic nucleus and observed the distribution of labelled perikarya within the cortex. The results obtained are at slight variance with the data presented here: while he also reports that infralimbic cortex projects largely to MDm, it is indicated that prelimbic and anterior cingulate cortices also project reciprocally to this region. In contrast, the major thalamic projection of prelimbic cortex was found to be MDl in the present study, but a considerable projection was also observed in MDm, although not to as great an extent as in MDl. Anterior cingulate cortex was found to have its main projection to AM and AD thalamic nuclei with little or no labelling in MD nucleus.

The reasons for these differences are not entirely clear although both WGA-HRP and HRP are thought to be able to diffuse a considerable distance away from the injection site (Gerfen et al., 1982) and may be taken up by damaged fibres or fibres of passage (Herkenham and Nauta, 1977; Gerfen et al., 1982). In contrast, injections of PhAL do not always fill all neurons within the injection site (Gerfen and Sawchenko, 1984). Therefore, in the study of Groenewegen extra neurons may have been "recruited" while in the present set of experiments, some neurons may have been omitted so that either more or less labelling may have been observed. This seems somewhat unlikely as the number of neurons either recruited or omitted would tend to be fairly small and consequently would not be thought to alter the intensity of labelling to any great degree. It could be argued that the size or extent of the injection site itself

might affect the labelling patterns. The injection sites in Groenewegen's experiments tended to be quite large in several instances so that they covered more than one region of the MD nucleus, a factor which might account for the differences in the labelling patterns. This could also be the case in the present experiments where several injections were found to cover more than one cortical area, but a considerable number of injections were relatively pure in terms of the cortical area labelled with PhAL and these tended to display good correlation between the cortical area injected and the thalamic area receiving the largest projections.

A further possibility for the differences observed may be that projections from cortex to MDl may first travel through MDm so that the pattern of labelling demonstrated by Groenewegen might be due partly to fibres of passage in MDm.

#### Corticocortical projections

There was also a distinct correlation between injection site and terminal labelling in other cortical areas in the ipsilateral hemisphere. The results obtained here for prelimbic and anterior cingulate cortices confirm those of Beckstead (1979) and are similar to those demonstrated in the cat (Cavada and Reinoso-Suarez, 1981; Room et al., 1985).

#### Corticostriatal projections

It can be concluded from the data that there are three general types of prefronto-corticostriatal projections in the rat, one of which can be further divided into two subgroups, which are as follows:

- (i) bilateral projections to distinct patches from prelimbic and infralimbic cortices.
- (ii) bilateral projections to distinct districts of the matrix from anterior cingulate cortex. Medial precentral cortex also provides inputs to matrix but in distinctly different areas of striatum.
- (iii) (a) fairly light bilateral projections to both patch and matrix within distinct areas from both prelimbic injections and those which involved some infralimbic neurons and to a much lesser extent, medial agranular cortex (although this was only demonstrated in one case).  
(b) dense bilateral projections to distinct districts of striatum covering both patch and matrix compartments, the labelled terminals often surrounding several "holes" (i.e. areas with little or no labelling) which correspond to patches demonstrated by CaBP histochemistry. This pattern occurred mainly with injections into prelimbic cortex.

These results therefore confirm previous findings that prelimbic and anterior cingulate cortices project to patches (Gerfen, 1984) and matrix (Donaghue and Herkenham, 1983) respectively. However, additional patterns have been revealed such that the medial precentral area also projects predominantly to matrix, while infralimbic cortex projects to patches. Other patterns not demonstrated by previous studies were also discovered. Injections into both infralimbic and prelimbic cortices provide inputs to matrix as well as patches such that fairly light, even labelling occurred in both compartments within distinct areas

of the striatum. Further observations implied that prelimbic cortex alone gives rise to another pattern of termination whereby distinct areas of matrix and some patches are densely labelled whereas other patches within the same area are not, a phenomenon mentioned by Goldman and Nauta (1977) in their studies on monkeys.

#### Projections to contralateral cortex

Labelling patterns within the contralateral cortex were examined after injection into the corresponding area of cortex in the ipsilateral hemisphere. In most cases, labelling was observed throughout all laminae but to varying degrees such that some displayed distinct terminal labelling predominantly in the deeper laminae (V and VI), whereas others were observed to have more intense labelling in superficial laminae (II and III). A degree of correlation was found to exist between the striatal projections of prelimbic (and some infralimbic) cortex and the laminar projections of the homologous contralateral cortex. Injections into the prelimbic cortex which provide inputs to both patch and matrix (and to a less conclusive extent, those into infralimbic cortex) labelled terminals in the superficial laminae to a greater extent than deeper laminae in the contralateral prelimbic cortex, whereas injections which preferentially labelled afferents in striatal patches provided inputs to mainly deep laminae of the homologous contralateral cortex. These results tend to suggest that separate populations of prelimbic and, to a less conclusive extent, infralimbic cortical neurons which differ in the laminar distribution of their cortical afferents, project differentially to the striatal patch and matrix compartments.

The contralateral cortical labelling observed here confirms the findings of Ferino et al. (1987). They injected WGA-HRP into the contralateral medial prefrontal cortex and found labelled neurons in laminae II, III and V of the ipsilateral homologous cortex. Injection of the tracer into the ipsilateral striatum labelled neurons in lamina V, which is the lamina in which most injected neurons were situated in the present study. Wilson (1987) also observed that crossed corticostriatal neurons were situated in superficial V and deep III, while brain stem projecting neurons were situated mainly in deep V.

Royce and Bromley (1984) have suggested that the occurrence of corticostriatal neurons in several laminae may not represent a non-specific distribution. Strong support for this view comes from several sources (Wilson, 1986; 1987; Ferino et al., 1987; Jinnai and Matsuda, 1979; Landry et al., 1984; Donoghue and Kitai, 1981; Royce, 1983), such that it is now believed that layer V brainstem projecting neurons which send collaterals to the striatum in the course of their passage into the internal capsule may be further divided into two groups. The first group comprises those with axon collaterals which extend over 1mm or more horizontally but are almost completely restricted to lamina V (Landry et al., 1984). The other group is made up of a separate population of corticostriatal neurons which are situated in lamina VI. Some of these neurons may be corticothalamic with axon collaterals that project to the striatum (Royce, 1983). Both of these groups are distinct from a much larger population of corticostriatal neurons which are located in the superficial part of lamina V and which do not project to thalamus or brainstem. A subset of this population has been shown

to project to the contralateral striatum (Wilson, 1987), although these neurons have been shown not to project to brainstem or thalamus (Catsman-Berrovoots et al., 1980; Miller, 1975). Consequently, it is unlikely that much of the input to the ipsilateral striatum observed in the present study came from brain stem projecting neurons as the extent of labelling in the contralateral striatum in all cases was almost as intense as that in the ipsilateral striatum.

Most perikaryal labelling in this study tended to be in the region of lamina V, with several cases displaying some labelling in more superficial laminae (especially III). It was impossible to differentiate between deep III and superficial V in most cases, but labelling of neurons which were distinctly situated in lamina III was found after some prelimbic injections and gave rise to labelling in both patch and matrix compartments surrounding "holes" of little or no labelling.

Ferino et al. (1987) also indicated that some cortical neurons within the superficial part of layer V and deep III project to contralateral cortex, contralateral striatum and ipsilateral striatum in any combination of the three by way of axon collaterals. This finding was supported by Wilson (1987). It is therefore proposed on the basis of the present results, that this group of crossed corticostriatal neurons can, like brainstem projecting neurons, be subdivided into two groups:

- (i) those in the superficial part of lamina V which might project preferentially to patches
- (ii) those in lamina III, projecting preferentially to matrix.

This scheme would account for the observation that both prelimbic and infralimbic cortex project to patches and matrix,



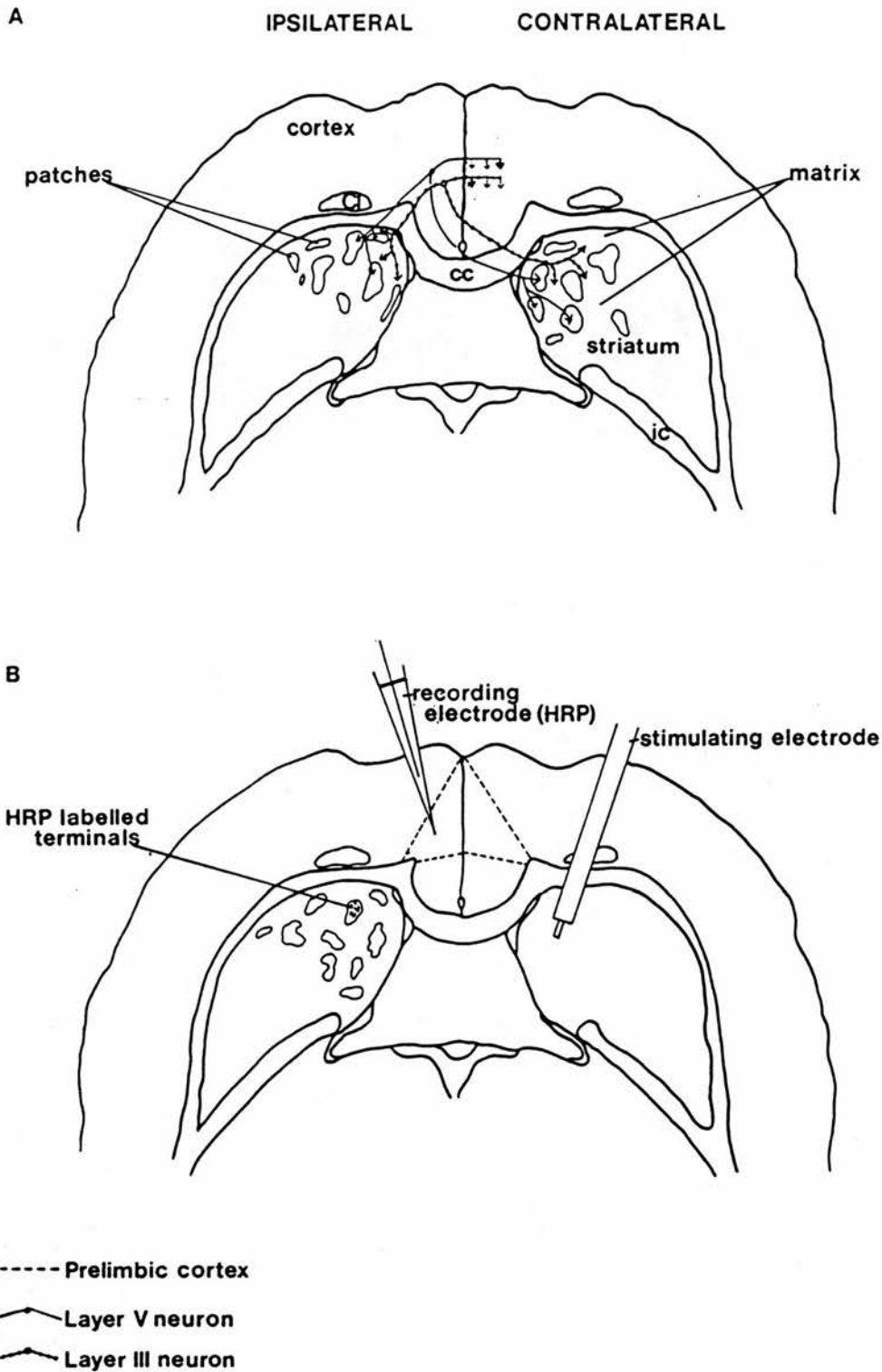


FIGURE 25: Possible organisation of the prelimbic cortical innervation of striatum and proposed experimental investigation.

- A. Proposed prelimbic innervation of striatal patch and matrix compartments. Layer V and layer III neurons may project preferentially to patches and matrix respectively.
- B. Possible experimental approach to explore the proposal outlined in A. The procedure is discussed in the text.

lamina V neurons (superficial) providing inputs preferentially to patches, while deep III neurons are responsible for projections to the matrix. This organisation is summarised in Figure 25A.

Those cases in which more superficial neurons are labelled and which display type (iii)(b) of striatal labelling might be due to the superficial neurons in lamina III (ie not deep III) projecting preferentially to matrix. This would produce dense and more widespread labelling in striatum so that additional areas of matrix would be labelled whereas additional patches would not, thus creating the "holes" observed in some cases.

Evidence to support this proposal comes from developmental studies. It has been known for a long time that cortex develops from the inside out (Berry et al, 1964). In other words, the deepest laminae are formed first followed by intermediate and then superficial laminae last. It has also been shown that neurochemically distinct patches are thought to appear at an earlier stage in development than matrix (Graybiel and Hickey, 1982)). This could therefore mean that as cortex develops, early corticostriatal projections are preferentially directed towards DA- and AchE- poor patches, while later and more superficial cortical cells would be directed towards matrix.

From the present data it cannot be determined whether the above proposal is correct due to the large numbers of perikarya stained in each cortical area and also because of the difficulty in determining the boundary between deep III and superficial V laminae. However, a combined electrophysiological and anatomical study might go some way to elucidating the situation (see Figure 25B). Cortical neurons could be antidromically fired from

contralateral cortex and/or contralateral striatum. Once identified in this manner they would be filled with HRP. The brain could then be cut into sections and adjacent sections stained for either HRP or CaBP. If it were possible to trace the terminals of the neuron into the ipsilateral striatum then it would be possible to determine if that particular cortical neuron projected to either patch or matrix (as shown by staining for CaBP immunoreactivity). If only one neuron per case was filled then it would be easier to determine its laminar origin.

#### Other systems

Thalamic, retrorubal and ventral tegmental inputs to the striatum have been found to ramify within the matrix (Herkenham and Pert, 1981; Herkenham et al., 1984; Kalil, 1978), whereas the substantia nigra projects in a heterogeneous manner to the rat striatum (Wright and Arbuthnott, 1981). Some cells located in the dorsal tier of the SNc project to matrix while others in the ventral tier project to patches. An additional set of substantia nigra neurons that are non-dopaminergic project to the matrix (Gerfen et al., 1987; Jimenez-Castellanos and Graybiel, 1987).

Similarly, earlier studies have demonstrated the existence of distinct patch and matrix projection systems (Gerfen, 1984, 1985; Gerfen et al., 1985). Matrix neurons project to the SNr except to those parts which contain DA neurons, whereas striatal neurons within patches not only project to locations of cell bodies and proximal dendrites of dopaminergic neurons in the ventral tier of the SNc but also those in SNr. A comparison of striatonigral organisation with the distribution of nigrostriatal projections to

either patches or matrix suggests that there are reciprocal as well as non-reciprocal relationships.

These earlier studies coupled with the results of the present study demonstrate that the prelimbic and infralimbic cortices and the substantia nigra may be the only major afferent sources of inputs to neurochemically distinct patches within the striatum (although a recent study carried out by Ragsdale and Graybiel (1988) demonstrated that fibres from the amygdala also selectively innervate patches). It has been suggested that, because mesolimbic DA neurons in the ventral tegmental area (VTA) project densely to the prelimbic cortex (Berger et al., 1976; Swanson, 1982), but sparsely to striatal opiate receptor patches (Herkenham et al., 1984), the prelimbic cortex may be a relay for mesolimbic influences such that they might converge with nigral dopamine projections in the patches. However, in the light of the present results where both prelimbic and infralimbic cortices have been found to project to patches and matrix, the VTA influences may also be allowed to converge with infralimbic inputs within patches, as well as other cortical inputs, and non-dopaminergic and dopaminergic projections from the SNr and SNc respectively.

The nature of the afferent input to the patches has been shown to be maintained by intrinsic striatal organisation. The dendrites of spiny neurons in patches are contained mainly within the patch boundaries (Herkenham et al., 1984; Penny et al., 1984) (although Bolam et al (1988) recently presented evidence to suggest that this is not always the case and see Chapter 1) and most of both patch and matrix neurons have local axon collaterals which appear to spread out only in their own dendritic domain (Preston et al., 1980; Wilson

and Groves, 1980). A small population of somatostatin-containing interneurons has been demonstrated (Gerfen, 1984) and has been suggested to provide a unidirectional link from patches to the surrounding matrix. Therefore, the patches could represent areas where inputs from one or two areas spread out through the striatum, interacting with inputs from other areas. However, in the light of the present results, the role of patches does not seem so clear cut, bearing in mind that both prelimbic and infralimbic cortices provide inputs to both patch and matrix.

#### Functional implications

Despite accumulating evidence that the patch-matrix system sets up particular input-output channels in the striatum (Gerfen, 1984) and that these have characteristic neurotransmitters associated with them, the exact function of this arrangement is still to be elucidated.

Evidence to suggest that there is a functional heterogeneity within the striatum comes from various sources. Divac (1980, 1983) showed that penicillin-induced epileptic foci in the cerebral cortex selectively co-activated associated neostriatal regions. Using autoradiography following administration of 2-deoxy-[ $^{14}\text{C}$ ]glucose, he demonstrated that this coactivation appeared in patches in the rat striatum and either spread gradually to the surrounding matrix or did not involve the matrix for tens of minutes.

Electrophysiological experiments carried out in primates have also demonstrated a distinct clustering of units with responses related to somatic sensory and motor properties (Alexander, 1984; Crutcher and DeLong, 1983; Liles and Updyke, 1985). Apart from one

brief report on glucose utilisation, none of these experiments have attempted to correlate these functional units with neurochemically distinct patches. However, a recent study carried out by Malach and Graybiel (1986) went some way to evaluating such possible structure-function correlations by exploring the intrastriatal distribution of functionally identified corticostriatal afferents from the primary somatosensory cortex in the cat, and related it to the patch-matrix system. It was found that these afferents were arranged according to a coherent body map in which rostral body parts were represented more laterally than caudal body parts. A surprising finding was that single cortical loci innervated striatal zones which were reminiscent of patches in terms of their shapes and sizes but they lay strictly within the striatal matrix. Malach and Graybiel suggested that this arrangement might allow for computations requiring cross-modality comparisons within the framework of an overall somatotopy, and in general striatal terms might set constraints on the nature of associative processing. However, this type of pattern has yet to be demonstrated in other striatal regions with inputs from other cortical areas.

With this demonstration of a patch-like organisation of the matrix, the question arises as to whether the patch-like terminal labelling observed in the present study might be within the matrix rather than overlapping with neurochemically defined patches. This seems unlikely as the sections were compared to adjacent sections stained for CaBP-immunoreactivity which has been shown to preferentially label the matrix (Gerfen et al., 1985). However, with the demonstration that prelimbic and infralimbic cortices project to both patch and matrix, it would be of interest to

determine the nature of the matrix projection from these areas in terms of whether or not they also conform to a patch-like pattern within the matrix itself. This remains to be elucidated, but may offer a clearer insight into the functional implications of compartmentalisation within the striatum.

#### SUMMARY

1. Corticostriatal terminals are distributed in distinct compartments which coincide with the neurochemically distinct compartments intrinsic to the striatum.
2. Both anterior cingulate and medial precentral cortex provide inputs to the matrix compartment but in distinctly different areas of striatum.
3. Injections into infralimbic cortex, as well as prelimbic cortex gave rise to labelling in patches.
4. Corticostriatal projections from the prelimbic cortex (and possibly infralimbic cortex) provide inputs to both striatal compartments in a manner which suggests different laminar origins for the patch and matrix afferents.

### CHAPTER 3

#### An Extracellular Electrophysiological Study of the Corticostriatal Pathway 'In Vitro'



## INTRODUCTION

The first successful electrophysiological demonstration of the existence of a corticostriatal pathway came from the strychnine neuronography experiments of Dusser de Barenne et al (1938, 1942). However, almost 20 years elapsed before gross evoked potentials were recorded within the striatum following peripheral stimulation in cats (Albe-Fessard et al., 1960a). These authors observed long-latency, large amplitude potentials evoked by various peripheral stimuli, but also noted that these responses lacked a somatotopic arrangement and could not be modified by extensive ablation of cerebellar and cerebral cortex. A subsequent study in which activity was recorded in response to stimulation of several cortical sites (Albe-Fessard et al., 1960b) demonstrated that heterogeneous afferents converged on one and the same striatal neuron. These results were confirmed by Laursen (1961) using a multilead microelectrode which allowed for precise recordings of individual units and definition of the limits of the spread of stimulating current.

A more detailed analysis of unitary activity was carried out by Roche Miranda (1965) on encephale isole, as well as chloralose- and nembutal-anaesthetised cats. Extracellular activity was recorded following stimulation of the neocortical surface and periphery. The most common response obtained was one action potential per stimulus with multiple spikes occurring only rarely. Some cortical regions such as the sigmoid and cingulate gyri gave rise to inhibitory effects upon stimulation.

Confirmation of these findings come from Sedgewick and Williams

(1967) although they also found that the inhibitory effects observed on spontaneously active neurons were difficult to interpret using extracellular recording techniques. A further observation of these authors was that the response of intracellularly recorded striatal neurons to cortical stimulation was composed of an excitatory phase followed by a period of inhibition, which the authors assumed came from a source outwith the striatum.

### Intracellular analysis

Improvements in intracellular recording techniques led to the demonstration by various groups of the response of single striatal neurons to cortical stimulation 'in vivo'. All these studies confirmed the initial findings of Sedgewick and Williams (1967), that the response consisted of two components which were referred to as excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs respectively). In general the EPSPs were observed as short latency membrane depolarisations lasting 5-50ms, while IPSPs were longer latency, shallow hyperpolarisations lasting from 80-300ms. The presence and properties of this type of response was fairly consistent between species (Buchwald et al., 1973; Hull et al., 1973; Kocsis et al., 1976; Matsuda and Jinnai, 1980; Herrling and Hull, 1980; Blake et al., 1976; Morris et al., 1979; Herrling, 1984; Vandermaelen and Kitai, 1980; Spencer, 1976; Bernardi et al., 1976a; Wilson et al., 1983a; 1983b; Bernardi et al., 1975; Wilson, 1986; Ryan et al., 1986), and has been observed after stimulation of other major striatal input systems such as those from thalamus (Buchwald et al., 1973; Hull et al., 1973; Kocsis et al., 1976; Sugimori et al., 1978; Vandermaelen and Kitai, 1980; Matsuda and Jinnai, 1980;

Purpura and Malliani, 1967; Kitai, Kocsis and Wood, 1976a; Wilson et al., 1983), substantia nigra (Buchwald et al., 1973; Hull et al., 1970; 1973; Park et al., 1980; Kocsis et al., 1976; Vandermaelen and Kitai, 1980; Matsuda and Jinnai, 1980; Frigyesi and Purpura, 1967; Kitai et al., 1975; Malliani and Purpura, 1967; Kocsis and Kitai, 1977; Kitai, Sugimori and Kocsis, 1976; Wilson et al., 1982) and raphe nucleus (Park et al., 1982; Vandermaelen et al., 1979). Similar observations have been made after local stimulation within the striatum (Marco et al., 1973; Marco, Copack and Edelson, 1973). The monosynaptic nature of this response was also demonstrated by various authors on the basis of its constant latency, despite changes in stimulus intensity or frequency (Kitai et al., 1976; Kocsis and Kitai, 1977; Blake et al., 1976).

Buchwald et al (1973) demonstrated that the EPSP-IPSP sequence was the predominant response of striatal neurons to cortical stimulation, occurring in 73% of the striatal neurons tested. A further 10% of the tested neurons responded with an IPSP only. Considering this and earlier anatomical findings (Kemp and Powell, 1971), Buchwald et al described the striatum as being comprised of inhibitory interneurons whose input from the cortex is excitatory and that the observed inhibitions were due to the simultaneous activation of neighbouring neurons which in turn inhibited each other. Confirmation for the role of inhibitory interneurons in the inhibitory phase of the response to cortical stimulation came from the work of Hull et al (1973) on cats. In these experiments a test stimulus was applied which was timed to produce a second excitatory-inhibitory response at various intervals after that evoked by a previous conditioning stimulus. Corticostriatal

excitation appeared to be almost totally insensitive to inhibition, while nigraly-evoked excitation was not. This led the authors to suggest that these responses might arise from proximally located synaptic contacts while both inhibitory and intrinsic synaptic contacts and inhibition-sensitive excitatory afferent synapses could be located more distally. The observations made in these studies, that the majority of striatal neurons have very little spontaneous activity, fitted well with the concept of many inhibitory interneurons.

Further evidence for a separate origin of the two components of the EPSP-IPSP sequence comes from the intracellular analysis of their development in cats. Several studies have indicated that striatal neurons respond to activation of their major inputs shortly after birth (Lidsky et al., 1976; Morris et al., 1977). Morris et al (1979) also demonstrated that while the EPSP is observed very early in development, the IPSP is not well-developed in young kittens, and only reaches adult values in terms of frequency of occurrence, amplitude and duration after 40 days. The implication of these results is that, assuming intrinsic mechanisms were responsible for the IPSP, they would not be well-developed during early postnatal periods in the cat.

#### Nature of the IPSP

The nature of the hyperpolarising component of striatal responses to cortical stimulation is somewhat controversial. Besides the studies of Buchwald et al. (1973) and Hull et al (1973) several other similar reports have provided evidence to suggest that inhibitory interneurons are involved (Vandermaelen and

Kitai, 1980; Kocsis and Kitai, 1977). Wilson et al (1983) on the other hand, suggested that cortical disfacilitation is largely responsible for IPSPs in striatal neurons. According to their hypothesis, the cortex provides an excitatory input which gives rise to a tonic depolarisation of striatal neurons above their true resting potential. When the cortex is inhibited by its afferents 'in vivo', striatal neurons lose their tonic excitatory input which leads to a hyperpolarisation and a return to the true resting potential. One prediction of their theory was that cortical ablation would lead to the disappearance of the IPSP component, and they were able to demonstrate some experimental support for this. Wilson (1981), in contrast, showed that after ablation of rostral cortex, intracellular responses to thalamic stimulation were obtained which were characterised by the presence of large amplitude IPSPs. Other authors have come down in favour of inhibitory collaterals of neostriatal projection neurons being responsible for the IPSP (Kocsis et al., 1976; Park et al., 1980) after the proposals that many striatal medium spiny neurons do in fact have axons terminating outside the striatum rather than intrinsically as was previously thought (Grofova, 1975; DiFiglia et al., 1976).

The experiments carried out by Park et al (1980) revealed the presence of recurrent autaptic inhibition of a much shorter duration than that usually described for inhibition of striatal neurons. The time course for shunting of nigraly-evoked EPSPs was shown to last for less than 35msec. Furthermore, it was found that systemic application of bicuculline, a GABA antagonist, blocked this inhibition, which led to the suggestion that it was the GABAergic striatal output neurons which were inhibiting themselves by way of

axon collaterals. Other 'in vivo' experiments in the rat (Bernardi et al., 1975; 1976b) demonstrated that GABA was the transmitter responsible for the long duration IPSP although drugs were administered systemically and so would exert relatively non-specific effects on the striatum. By the more direct approach of microiontophoretic application of bicuculline methochloride to cat striatal neurons, Herrling (1984) was able to abolish the early part of the inhibitory phase leaving a residual hyperpolarisation which was unaffected by prolonged applications of the drug. In contrast, Wilson and Wilson (1985) observed a complete block of evoked hyperpolarisation after both systemic and local application of baclofen.

Striatal IPSPs therefore appear to be comprised of two components, an early phase which is mediated by GABAergic mechanisms and a later phase, the nature of which remains unknown.

The use of the 'in vitro' slice technique devised by Yamamoto and McIlwain (1966) enables the striatum to be isolated from its inputs so that intrinsic properties alone can be studied together with their pharmacology. Local stimulation within the striatum has demonstrated an EPSP similar to those observed in 'in vivo' studies, while the IPSP, when present, is of short latency (3-38msec) (e.g. Kitai and Kita, 1984; Kita, Kita and Kitai, 1984, 1985a; Lighthall and Kitai, 1983; Lighthall et al, 1981; Cherubini et al, 1988). Lighthall and Kitai (1983) and Kita et al (1985b) have demonstrated unequivocally that this short duration inhibition is mediated by GABAergic neurons, by both application of bicuculline and picrotoxin. The responding neurons were intracellularly labelled with HRP and exhibited morphological characteristics of medium spiny

neurons, thus leading the authors to suggest that the inhibition was mediated by GABAergic axon collaterals.

The fact that only the short duration IPSP is observed in slice preparations implies that the source of the long duration IPSP or part of it, is probably extrinsic to the striatum as Wilson et al (1983) suggested. Their cortical disfacilitation mechanism is the only one proposed to date and provides a very viable suggestion for the source of the long-lasting IPSP.

#### Nature of the EPSP component

While much controversy has surrounded the nature of the IPSP component the elucidation of the physiology and pharmacology of the EPSP has been less difficult. The mechanism by which cortical inputs directly activate striatal neurons has been the subject of several electrophysiological, biochemical and pharmacological experiments.

The electrophysiological experiments of Spencer (1976) provided the first evidence that the transmitter involved in the corticostriatal pathway could be an excitatory amino acid. These showed that the GLU antagonist glutamic acid diethyl ester (GDEE) could block the excitatory activity induced in striatal neurons by cortical stimulation, as well as depressing the excitation produced by iontophoretic application of GLU, aspartate (ASP) and homocysteic acid. A similar study was carried out by Stone (1979) using the excitatory amino acid antagonist D, L- $\alpha$ -aminoadipic acid. This antagonist had been claimed to show a preference for blocking excitation produced by ASP rather than GLU (Bisco et al, 1977).



However, Stone demonstrated that iontophoretic application of the antagonist showed no selectivity for either amino acid in 70% of the neurons tested, but did antagonise the effects of both, again suggesting the involvement of these amino acids in corticostriatal neurotransmission.

Support for these findings came from various 'in vitro' studies which investigated the changes in high affinity transport and release of GLU from striatal preparations following the destruction of the cortico-striatal pathway (Divac et al, 1977; Kim et al, 1977; McGeer et al, 1977; Reubi and Cuenod, 1979; Fonnum et al, 1981; Sandberg et al, 1985; Roberts et al, 1982). Further confirmation also came from 'in vivo' studies using microdialysis (Young et al, 1986) and the push-pull cannula method (Girault et al, 1986) to study release of GLU and ASP from the rat striatum after cortical lesioning and potassium-stimulation respectively.

Following on from earlier electrophysiological studies, intracellular recording techniques were employed to look at the effects of microiontophoretic application of excitatory amino acids on striatal neurons (Bernardi et al, 1976a; Herrling and Hull, 1980). Unfortunately, only the effects of L-GLU were examined and no antagonists were used. Since then, however, the availability of a number of specific excitatory amino acid agonists and antagonists has led to the postulation of three types of receptors for excitatory amino acids: quisqualate (QA), kainate (KA) and N-Methyl-D-Aspartate (NMDA) (Watkins, 1981). This led to the subsequent biochemical demonstration of some of these receptors within the striatum (Scatton and Lehman, 1982), which in turn prompted various studies to be carried out to determine the



mechanism by which excitatory amino acids activate striatal neurons, and hence the pharmacology of the responses to cortical stimulation.

Herrling et al (1983; 1985) looked at the effects of microiontophoretically applied agonists and antagonists of excitatory amino acid receptors on cortically evoked synaptic potentials in anaesthetised cats. They concluded that the cortically-evoked monosynaptic EPSP is probably mediated by QA or KA receptors as selective NMDA antagonists had failed to block the EPSP.

Local stimulation of striatal slices maintained 'in vitro' has also been shown to evoke an EPSP although controversy surrounds the transmitter involved. Misgeld and colleagues (1982; 1980; 1979) and Doht and Misgeld (1986) report the presence of only cholinergic mediated EPSPs within striatal slices. However, studies carried out by Cordingley and Weight (1986) and Cherubini et al (1988) found responses identical to those obtained for cortically evoked EPSPs 'in situ' (Herrling et al, 1983; Herrling, 1985) with respect to their pharmacology, even though the origin of the synaptic terminals responsible for intrastrially evoked EPSPs in a slice preparation cannot be determined.

#### Topography of the corticostriatal pathway

While there have been numerous studies carried out on the electrophysiological properties of cortically activated striatal neurons, very few have provided detailed descriptions to indicate whether or not the responses are localised within certain regions of the striatum. This seems paradoxical in view of the great number of anatomical studies carried out to investigate the problem (e.g. Goldman and Nauta, 1977; Goldman-Rakic and Selemon, 1985; Donoghue

and Herkenham, 1986). However, several studies have been carried out in various species in an attempt to redress this situation.

Experiments carried out by Liles (1973; 1974) in which evoked potentials were examined in *cereau isole* cats revealed that connections were organised in a mediolateral topographic arrangement originating from the anterior cortical regions and cingulate gyrus, but a functional projection from parietal cortex to the striatum was not demonstrated. Blake et al (1976) on the other hand, did find that evoked potentials were transmitted from more posterior regions of cortex, with some topographic segregation of anterior and posterior projections being observed. However, no evidence of a mediolateral organisation of inputs was apparent.

An attempt to examine the reliability of evoked potentials in demonstrating the topographical details of the corticostriatal projection was made by Updyke and Liles (1987) who compared them to autoradiographically labelled axon terminals in cats. They claimed that their data indicated a close relationship between the distribution of striatal evoked potentials to stimulation of specific areas of motor cortex and the distribution of axon terminals from the same cortical site. However, the method used seemed somewhat crude; each recording track was labelled with [ $^3\text{H}$ ] proline after withdrawal of the stimulating electrode. It was apparent that the resulting injection site would be much larger than the diameter of the stimulating wire, so the reliability of evoked potentials in demonstrating the topographical details of the corticostriatal projection remains unclear.

Microstimulation experiments have also been carried out on monkeys (Liles and Updyke, 1985; Crutcher and DeLong, 1984;

Alexander and DeLong, 1985a; 1985b) which have led to the speculation that functional equivalents of the distinct neurochemical compartments observed within the striatum of various species (e.g. Graybiel and Ragsdale, 1981; 1982; Graybiel et al, 1981) were present in the monkey putamen. These experiments were somewhat naive in their approach as current spread was very much an unknown factor in the results, as was the use of gross recording techniques.

One attempt to overcome these problems was carried out by Wilson et al (1983b) using cats. Intracellular responses to peripheral stimuli were studied to determine the degree to which neurons and/or regions of striatum receive different sensory input. A homogenous pattern of intracellular responses was observed in the head of the striatum, rather than the mosaic-like pattern predicted by anatomical studies. The authors suggested that this was probably due to the fact that peripheral stimulation would tend to activate other inputs to striatum which would therefore confuse the issue somewhat.

A great deal more successful were the experiments carried out by Richards and Taylor (1982) in rats and Malach and Graybiel (1986) in cats. In both cases, somatotopic body maps were demonstrated within striatum after sensory stimulation, while Malach and Graybiel observed clustering of responding neurons within specific areas of the striatal matrix.

The slice technique of Yammamoto and McIlwain (1966) offers a useful way of studying the striatum 'in vitro' not least of all because it allows for easier placement and location of stimulating

and recording electrodes. Most 'in vitro' studies carried out to date have used local stimulation or have only examined intrinsic activity within a striatum isolated from extrinsic influences. Therefore, a slice preparation was developed in which it was hoped that the corticostriatal pathway would be preserved to enable it to be studied 'in vitro'. The aim of this study was thus to determine if corticostriatal connections were maintained in the slices and if so to elucidate some of their properties using extracellular recording techniques.

## METHODS

### Animals

Male albino rats weighing 120-180g were used in these experiments.

### Slice chamber

The chamber used was a slight modification of the Andersen type of chamber (Langmoen and Andersen, 1981), and was manufactured within this department (Figure 26). It consisted essentially of a hollow perspex cylinder with a perspex lid which was usually kept secured tightly into place.

The lower half of the chamber was filled before each experiment with distilled water which was heated up by means of two heating elements inserted into the base of the chamber (labelled 2 in Figure 26). These elements were in turn connected to an external control box. The temperature of the water in the bath could be thermostatically maintained at an almost constant temperature due to the presence of a feedback probe within the bath which was also connected to the external control box. The temperature was usually maintained at  $36^{\circ}\pm 1^{\circ}\text{C}$  and was checked regularly by the use of a battery operated temperature probe, in order to ensure that optimum temperatures were being used. This was an important check to make due to the fact that at temperatures only a little above  $37^{\circ}\text{C}$  activity rapidly declines and the slices die.

A temperature of  $36-37^{\circ}\text{C}$  was chosen as this is the optimum temperature to observe "physiological" processes at work. It was felt that use of lower temperatures (e.g.  $32-34^{\circ}\text{C}$ ) would not allow

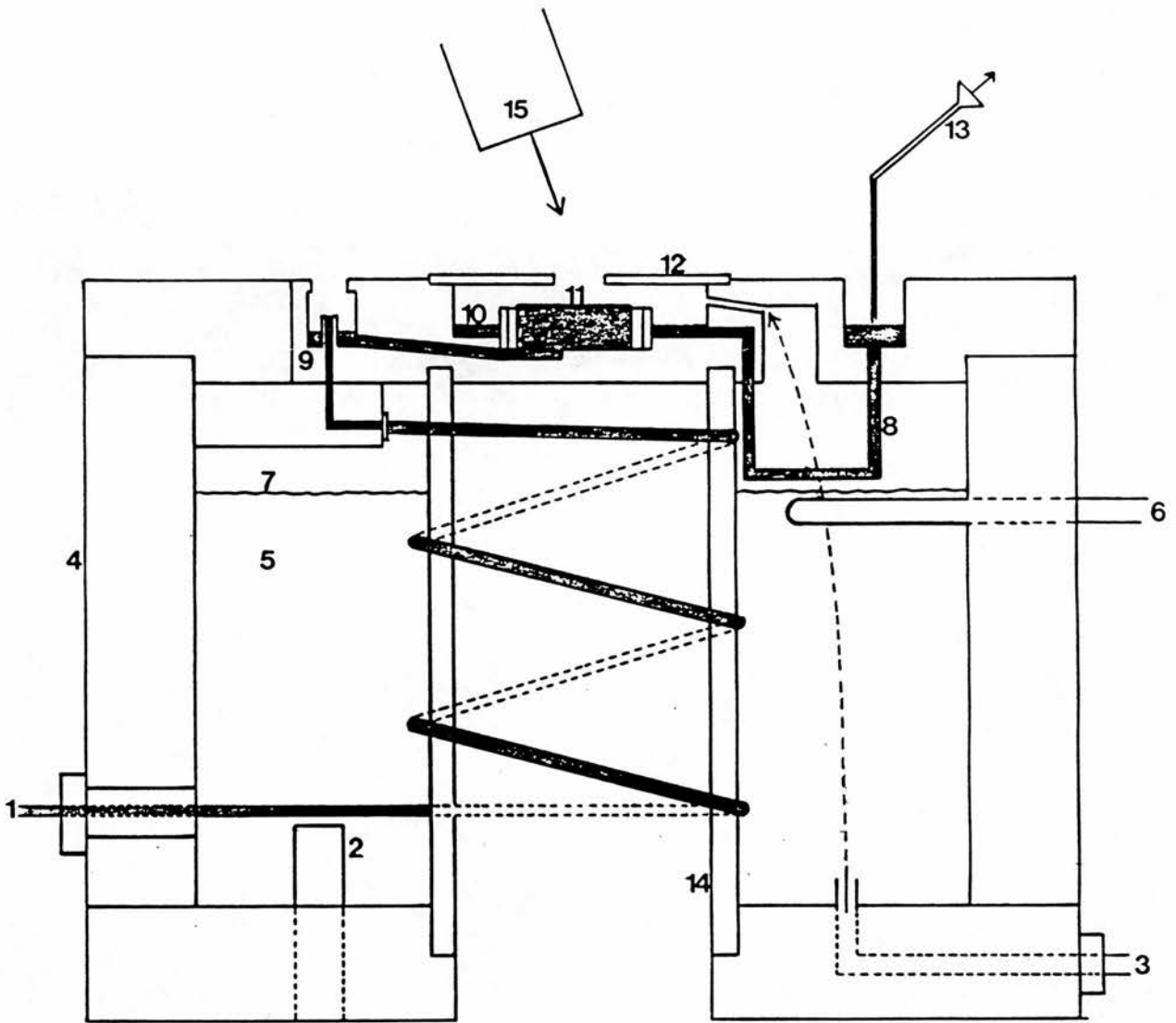


FIGURE 26: Diagram of the slice chamber.

Adapted from Langmoen and Andersen (1981).

- |                              |                                      |
|------------------------------|--------------------------------------|
| 1. ACSF inlet                | 9. bubble trap                       |
| 2. brass heating element (2) | 10. recording chamber                |
| 3. gas outlet (4)            | 11. nylon mesh on recording platform |
| 4. outer cylinder            | 12. removable lid                    |
| 5. inner chamber             | 13. suction pipette                  |
| 6. temperature monitor       | 14. inner cylinder                   |
| 7. water level               | 15. light source                     |
| 8. draining well.            |                                      |

for real physiological phenomena to be observed. Rather, other processes and events may come into being at these temperatures, and a general slowing of events would occur.

Four gas inlets were also present in the lower half of the bath(3). These were connected together by means of plastic tubing and "T" pieces, this "circular" tube in turn being connected to a cylinder of 95% Oxygen ( $O_2$ )/5% carbon dioxide ( $CO_2$ ). This allowed for the gas to be bubbled into the slice chamber, the rate of flow being controlled externally by a metal tube clamp. The gas travelled through the wall of the chamber by means of narrow tunnels cut through the perspex, and then out into the chamber via four aquarium gas filters. Once in the chamber the gas bubbled through the warm distilled water.

Another narrow inlet in the lower part of the chamber allowed fluid to be fed into the chamber via a narrow plastic tube(1). This tube was lightly coiled in the chamber so that adequate heat transfer from the warm distilled water to the fluid in the tube could occur. The length of tubing lying within the chamber was found to be crucial for adequate control of fluid flow.

The lid portion of the chamber consisted of a metal inlet tube fused into the perspex, to which the plastic inlet tube was attached, thus allowing the warmed-up fluid to travel into the lid portion and hence into the recording chamber. The metal inlet tube was in turn connected to a bubble trap cut into the perspex(9).

The presence of a bubble trap was necessary in order to prevent bubbles being allowed into the recording chamber. The appearance of bubbles under a slice tended to result in the slice being starved of medium. The bubble trap was connected to a small recording chamber

(approximately 3cm diameter)(10) in which a removable perspex ring was situated and held securely in place by a circular groove cut out in the base of the recording chamber. A piece of 20 denier nylon stocking was stretched across the top surface of the ring and held tautly in place by a slightly larger ring pushed over the smaller one. This therefore produced a small platform for slices to be placed upon(11).

Surrounding the top edge of the chamber were several holes (diameter 2-3mm) cut into the perspex of the lid. These holes allowed gases which were coming out of solution in the lower half of the main chamber to be transferred to the recording chamber, thus supplying slices with an adequately moist oxygenated atmosphere.

A small hole cut in the recording chamber base was connected to a 'U' shaped outflow tube(8). The whole system was securely fastened to a metal plate which sat on an inner tube in order to produce a "floating table" effect to minimise shock to the bath.

#### Bathing Medium

The standard medium used throughout the experiments was a modified Yamamoto's Medium. The composition was (in mM): NaCl 124, KCl 3.3,  $\text{NaHCO}_3$  26, CaCl 2.5,  $\text{MgSO}_4$  2.4,  $\text{KH}_2\text{PO}_4$  1.25, D-glucose 10. The pH of the solution was approximately 7.4 and was checked periodically with a pH meter.

During each experiment a reservoir of medium was kept at 36°C in a water bath, and bubbled constantly with the 95%  $\text{O}_2$ /5% $\text{CO}_2$  mixture. By bubbling gases into the medium, the pH of the solution was brought to approximately 7.4 and by the dissolving of these gases in the medium, the slices were supplied with the necessary



requirements for survival.

Initially the reservoir of medium was left at room temperature but this caused the problem of large gas bubbles accumulating in the inlet tubes of the chamber due to dissolved gases coming out of solution on being heated up within the chamber. If the build up was severe enough to prevent flow of the medium then the slices died. However, by adopting the procedure of keeping the fluid reservoir at approximately the same temperature as the fluid in the bath, dissolved gases were already at equilibrium and this prevented the above problems occurring.

From the reservoir, medium flowed through a plastic tube, under gravity, into a constant head device. This was essentially a 10ml syringe with a hole cut in the base through which the nozzle of a 1ml syringe was pushed. The 1ml syringe thus acted as an overflow pipe for excess medium, but the rate of flow of fluid into the constant head device was also controlled by means of a metal tube clamp attached to the outlet tube from the reservoir. This constant head device acted as a flow rate regulator in that it prevented any sudden surges of fluid into the chamber which might have caused the loss of penetrated cells during an experiment.

A thin plastic tube attached to the nozzle of the 10ml syringe carried the medium towards the chamber where it was fed through the wall via the inlet tube (labelled 1 in figure 26), through the heating chamber and up through the bubble trap. From there it travelled into the recording chamber via a hole drilled in the perspex. From the base of the recording chamber the fluid was forced up and out over the nylon mesh and over the top of the platform. Its movement was aided by the placement of four cotton

wool wicks placed over the edge of the platform. The medium was then allowed to escape through the 'U' shaped outlet tube and was aspirated from the chamber by a hypodermic needle attached to a rubber tube(13). A peristaltic pump was used to draw the fluid from the outlet tube. The medium was not recycled but was carried to a waste bottle.

#### Flow rate

A very critical parameter in the determination of slice survival was the rate of flow of the medium through the bath. If the flow rate was too high compared to the outflow then the recording chamber flooded; if it was too low, then the top surfaces of the slices would dry out and become more opaque due to insufficient perfusion. By altering the height of the constant head device, the height of the aspirator or the rate at which fluid was pumped out, the flow rate could be maintained relatively constant over an 8-10 hour period. The optimum flow rate was found to be approximately 1-2ml per minute and this was periodically checked to ensure constancy of conditions.

#### Preparation of slices

Rats were stunned by a sharp blow to the back of the neck and then decapitated. The severed head was placed cut surface down on to a paper towel to help drain blood away from the head, and the skin was cut away from the skull. The skull itself was then chipped away rapidly but carefully down to the snout using bone clippers. Once the dorsal surface of the brain had been exposed, the dura was cut carefully with fine scissors and the whole brain was levered out

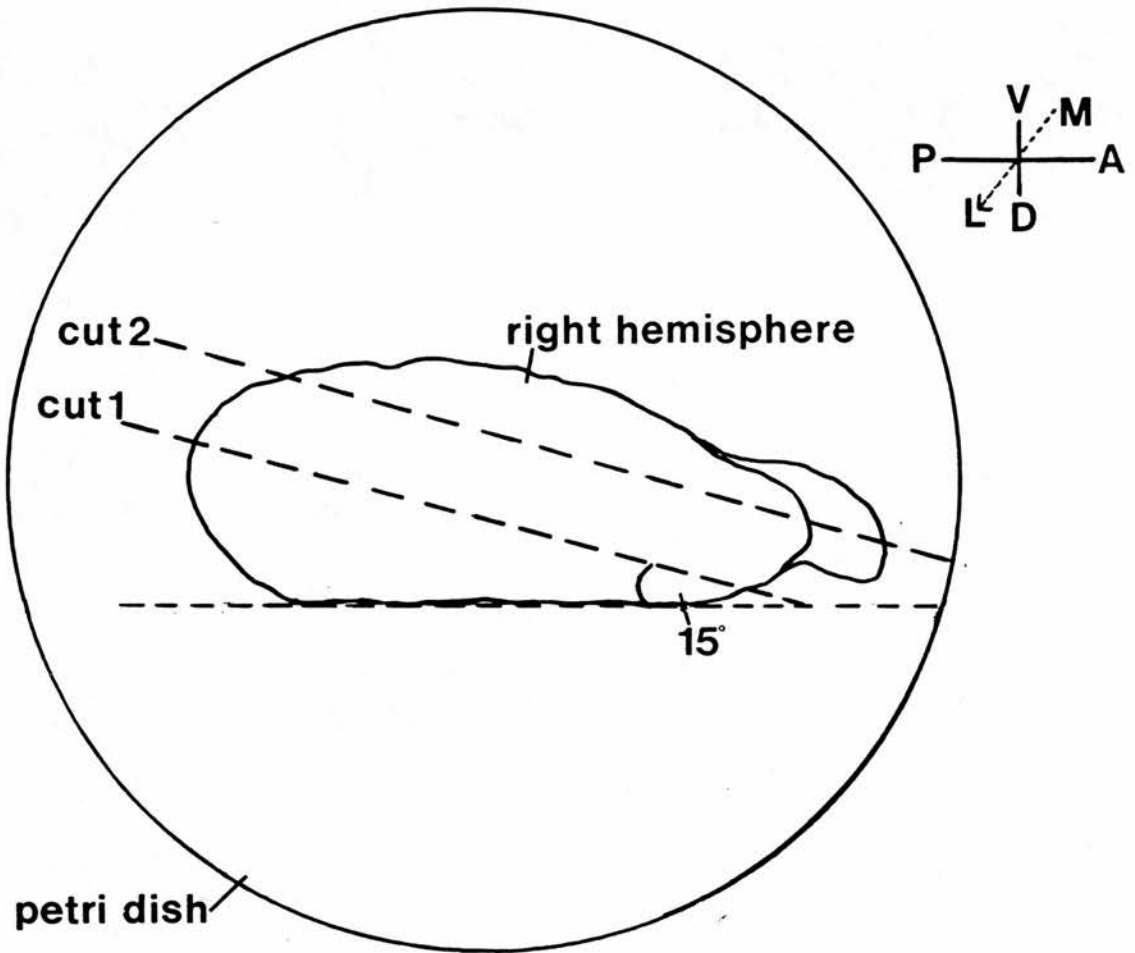


FIGURE 27: Production of a striatal tissue block.  
A description of the procedure is outlined on p.125.

Abbreviations:

A anterior  
D dorsal  
L lateral

M medial  
P posterior  
V ventral

with a microspatula, which was also used to sever the cranial nerves. The brain was then dropped into a beaker of ice-cold medium for several seconds before being placed on a petri dish covered with moistened filter paper.

A block of tissue was cut from the brain by first removing the cerebellum. The cerebral hemispheres then were separated using a single-edged razor blade to make one rapid clean cut. In most cases the right hemisphere was used to produce the slices.

Parahorizontal blocks of tissue approximately 5 mm thick were cut from one hemisphere at an angle of  $15^{\circ}$  to the cortical surface (Figure 27). The correct angle was obtained by lining up the hemisphere on to two lines drawn at  $15^{\circ}$  on the petri dish. The resulting block of tissue was then fixed to a teflon cutting block using cyanoacrylate glue (RS Components). Support was given to the back edge of the tissue block with respect to the direction of movement of the cutting block by pre-fixing a cube of 4% agar on to the cutting block and lining up the tissue block against it. Gentle pressure with a pair of blunt forceps made sure that the tissue was secured to the cutting block. Once secured, the tissue was immersed in oxygenated medium maintained at room temperature in the trough of a Vibroslice (Campden Instruments), which was used to section the blocks.

300 $\mu$ m thick slices were usually cut as this thickness appeared to be optimum for the particular type of recording chamber used and the experiments carried out. These sections extended almost the whole length of the brain in the rostro-caudal axis (figure 28). Consequently, any tissue not required in the experiments, such as the hippocampus, could be removed by carefully cutting with a fine

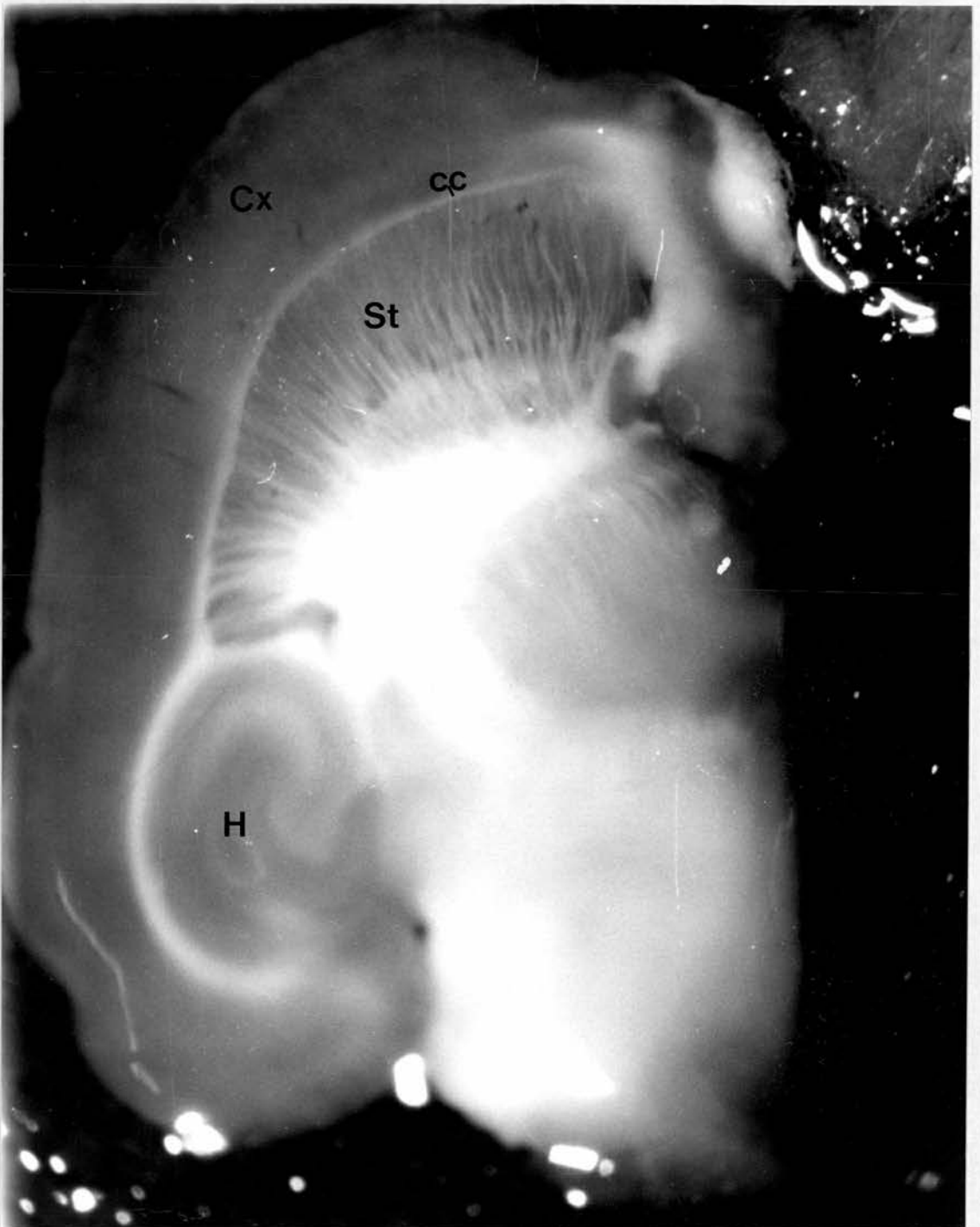


FIGURE 28: Photograph of a striatal slice.  
This was taken during the one hour incubation period  
prior to recording.

Abbreviations:

cc corpus callosum  
Cx cortex

H hippocampus  
St striatum

scalpel. However, this proved somewhat time-consuming and was avoided when possible.

The slices were then carefully placed in the recording chamber by first collecting them on a large flat spatula and then using a fine paint brush to gently sweep them off on to the platform in the bath. The slices did not sit directly on the nylon mesh but were placed on a piece of lens tissue which had been cut to fit on the mesh. Once the slices were in position a lid with a small hole in the middle was placed over the platform to help prevent them from drying out by retaining the gases and moisture within the chamber.

The tissue was always maintained in these conditions for approximately one hour prior to recording.

The whole procedure from killing to the placement of slices in the chamber took approximately 5 to 8 minutes. If for any reason the time taken was longer than this then the slices were discarded as experience showed that a longer preparation time generally resulted in unsuccessful or poor recordings.

### Electrical recordings

Striatal neurons were recorded extracellularly with glass microelectrodes (Clark Electromedical Instruments, GC150F-10) filled with 2% Pontamine Sky Blue in 0.5M sodium acetate (DC resistance 40-80M Ohms) or 3M KCl (30-70M Ohms). Electrodes containing the former solution were used in preference as the Pontamine allowed for easier visualisation and hence placement of the electrode tips.

Intact striatal fibre bundles were visible for several millimetres (figure 28) inwards from the edge of the callosum due to the angle at which the brain was cut. This allowed for the

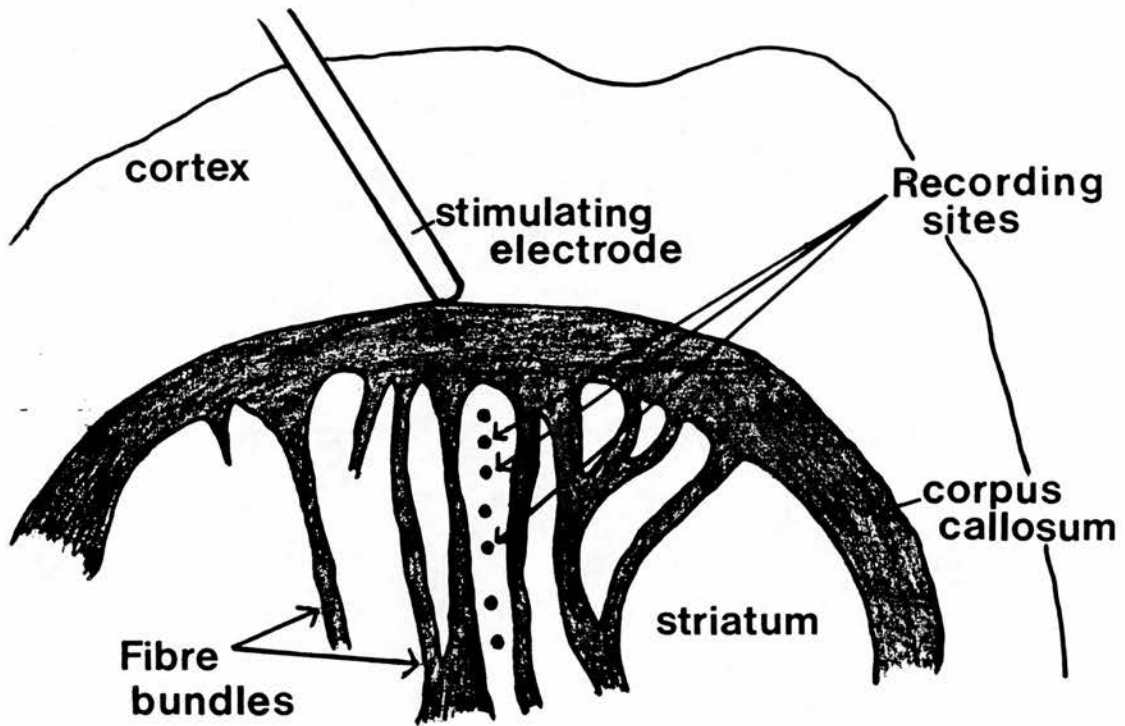


FIGURE 29: Relative positions of stimulating electrode and recording sites in a typical recording experiment.

placement of the recording electrode at different positions (approximately 50 $\mu$ m apart) within the grey matter along one side of the fibre bundle. By using a camera lucida drawing attachment on a dissecting microscope, recording positions were accurately plotted with respect to the fibre bundles and the stimulating electrode. Figure 29 illustrates an experiment of this type.

The recording electrode was connected by a silver wire to a WPI preamplifier. The output from the source was connected to a differential amplifier (Tektronix) of a dual beam storage oscilloscope (Tektronix). Cell activity was filtered ( -3dB at 500 Hz-10K Hz) and displayed on the oscilloscope. Under these recording conditions the noise level was usually around 50-100 $\mu$ V.

#### Stimulating electrodes

Stimuli (0.01-100V, 0.1ms pulse width, 0.77Hz) were applied to a stainless steel wire placed on the surface of a slice on the cortical side of the corpus callosum. Stimulation and timing were controlled by a Digitimer 4005 timing unit and an isolation unit provided timed, constant voltage pulses.

#### Measurement of firing frequency

The output of the differential amplifier was connected to a window discriminator (D130 - Spike Processor, Digitimer Ltd.) and the windows were displayed on the viewing oscilloscope. The spike processor was set to count the number of spikes crossing the window every minute. If possible, the firing rate of a particular neuron was monitored for at least 5-10 minutes.



Paired-pulse stimulation

In order to test the inhibitory phase of the cortical response, the intervals between pairs of cortical stimuli were varied over 1-150 msec. The stimulus intensity used was usually 1.5 times greater than that required to produce the initial response. The smallest interval between stimuli which still produced a second response was the value used in the analysis of the results.

## RESULTS

The data in this section is derived from extracellular recordings of 91 neurons from 24 rats and is summarised in table 4. All recorded neurons were located in the grey matter between the radially-oriented fibre bundles (or fascicles) coursing through the striatum. Several evoked potentials were also observed in some preparations, while one neuron yielded a brief intracellular recording (these are dealt with in more detail in Chapter 5). Several neurons were usually recorded in each experiment but up to 7 different neurons were recorded per track on occasions.

The majority of neurons observed in this study were completely silent during the experiments and fired only in response to cortical stimulation (see Table 4). Spontaneous activity was, however, recorded in 15.4% of the neurons. This was either seen as rapid burst-like pattern of firing (figure 30) or as random action potentials (or spikes). Only 2 neurons (2.2%) were unresponsive to cortical stimulation (Figure 31).

Those neurons which were spontaneously active and which fired rapidly in bursts usually had up to approximately 20 spikes within each burst. The mean firing rate of these neurons was  $2.84 \pm 2.18\text{Hz}$  ( $n = 7$ ) and the amplitude of the action potentials ranged between 2 and 15mV. Two neurons which exhibited random single spikes were also recorded. Their mean firing frequency was  $0.07 \pm 0.08\text{ Hz}$ . All the spontaneous activity observed did not appear to be confined to any one region of the striatum and was recorded at depths of 30–150 $\mu\text{m}$  below the surface of the slice.

TABLE 4. COMPARISON OF STRIATAL NEURON FIRING 'IN VITRO'  
AND 'IN VIVO'

Firing pattern	IN VITRO		IN VIVO	
	No.of cells	% of total	No.of cells	% of total
Completely silent	77	84.6	228	61
Bursts	11	12.1	88	16
Occasional < 0.1Hz	3	3.3	59	23

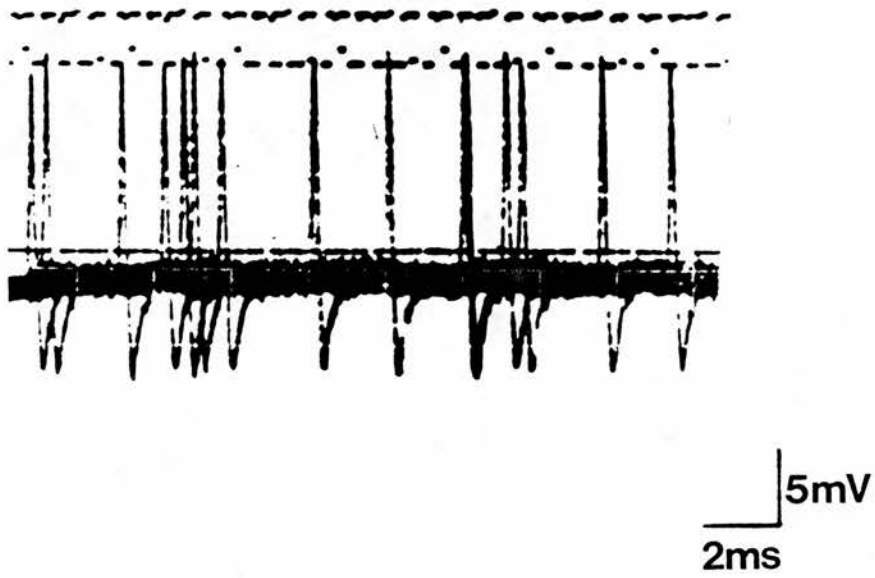


FIGURE 30: An example of spontaneous activity recorded from a striatal neuron.



FIGURE 31: Unresponsiveness of a spontaneously active striatal neuron to cortical stimulation.

The cortical stimulus is indicated by the arrow.

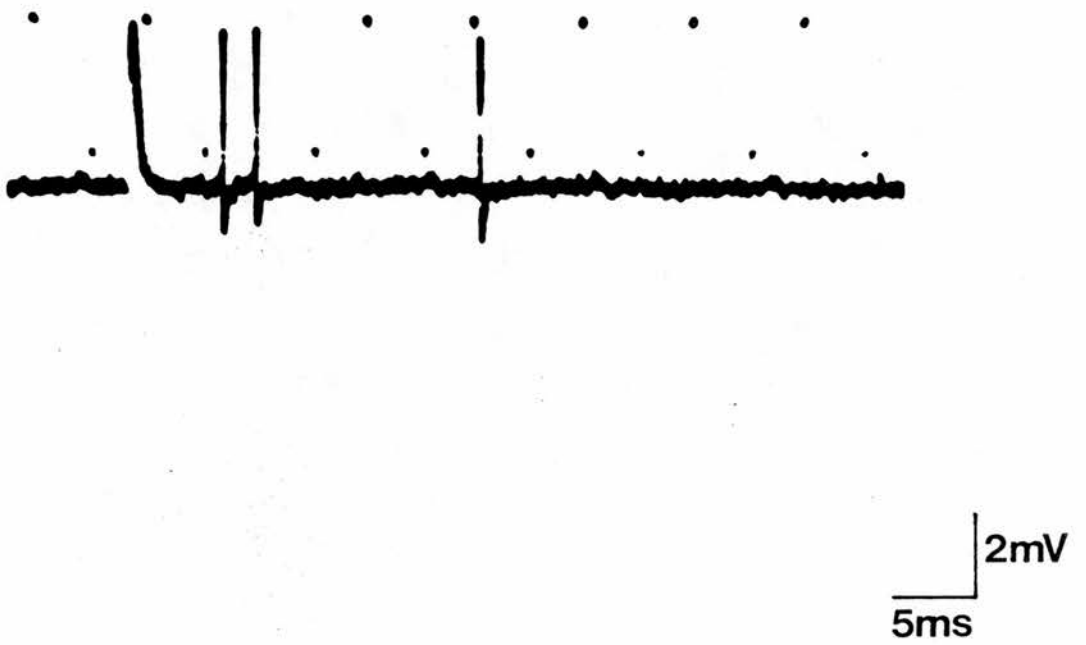
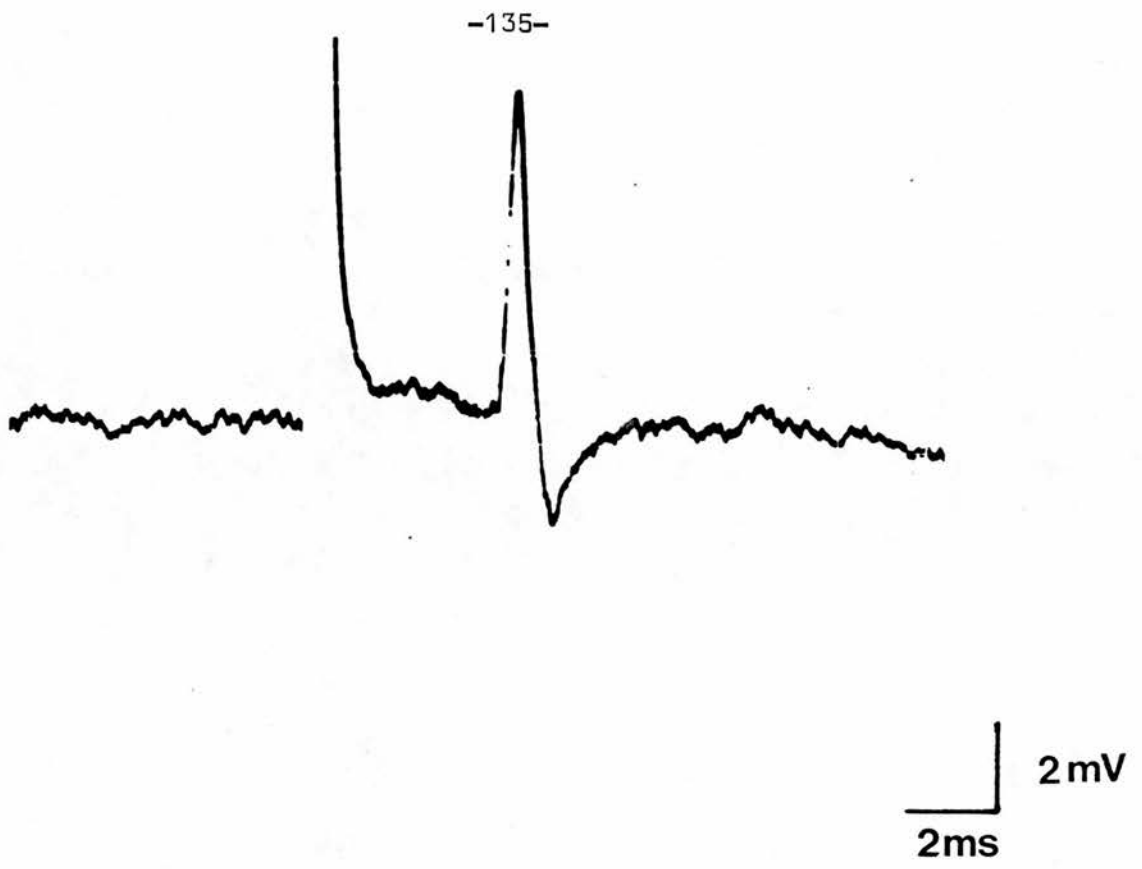


FIGURE 32: Cortically evoked action potentials from a striatal neuron.

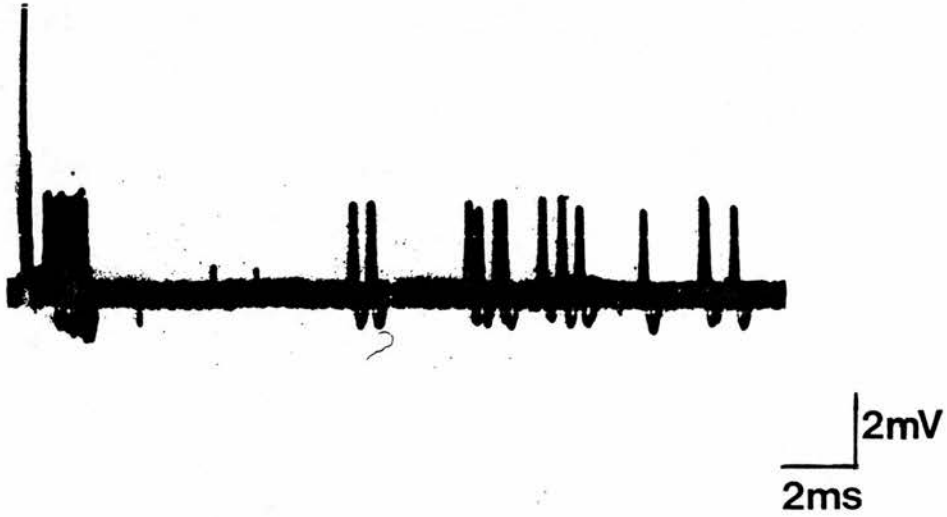


FIGURE 33: The initial excitation and subsequent inhibition of a spontaneously active striatal neuron by cortical stimulation.

The inhibition of spontaneous firing lasts approximately 17ms.

### Responses to cortical stimulation

Most striatal neurons (84.6%) were silent but could be excited by stimulation of the overlying cortical white matter (0.02 - 1.25mA, pulse width 0.1ms). The usual response of these neurons to cortical stimulation consisted of one spike per stimulus (Figure 32, top trace) although on rare occasions, two or three spikes per stimulus were observed (Figure 32, bottom trace).

At threshold levels of current (mean =  $0.74 \pm 0.33$ mA,  $n = 89$ ) the response latency usually varied slightly for each neuron with each stimulus. However, increasing the stimulus strength well above the threshold level usually led to the latency of the responses becoming more constant, but did not cause an overall change in the latency observed.

Of the fourteen spontaneously active neurons observed in this study, 12 could also be driven by cortical stimulation. The response consisted of an initial excitation followed by a prolonged inhibition in most cases (Figure 33), the period of this inhibition lasting for approximately 15-90ms. Usually the inhibition was followed by a rebound excitation. However, one of the spontaneously active neurons displayed a slightly different pattern of activation after cortical stimulation, namely a long initial inhibition which lasted for 80 ms, after which time excitation occurred. The initial excitation of the majority of the spontaneously active neurons responding to cortical stimulation was of similar latency to that of silent neurons. Figure 34 is a latency histogram for all cortically driven neurons. The mean latency was calculated to be  $4.35 \pm 3.35$ ms ( $n = 88$ ) with a range of values between 1.9 and 28ms.



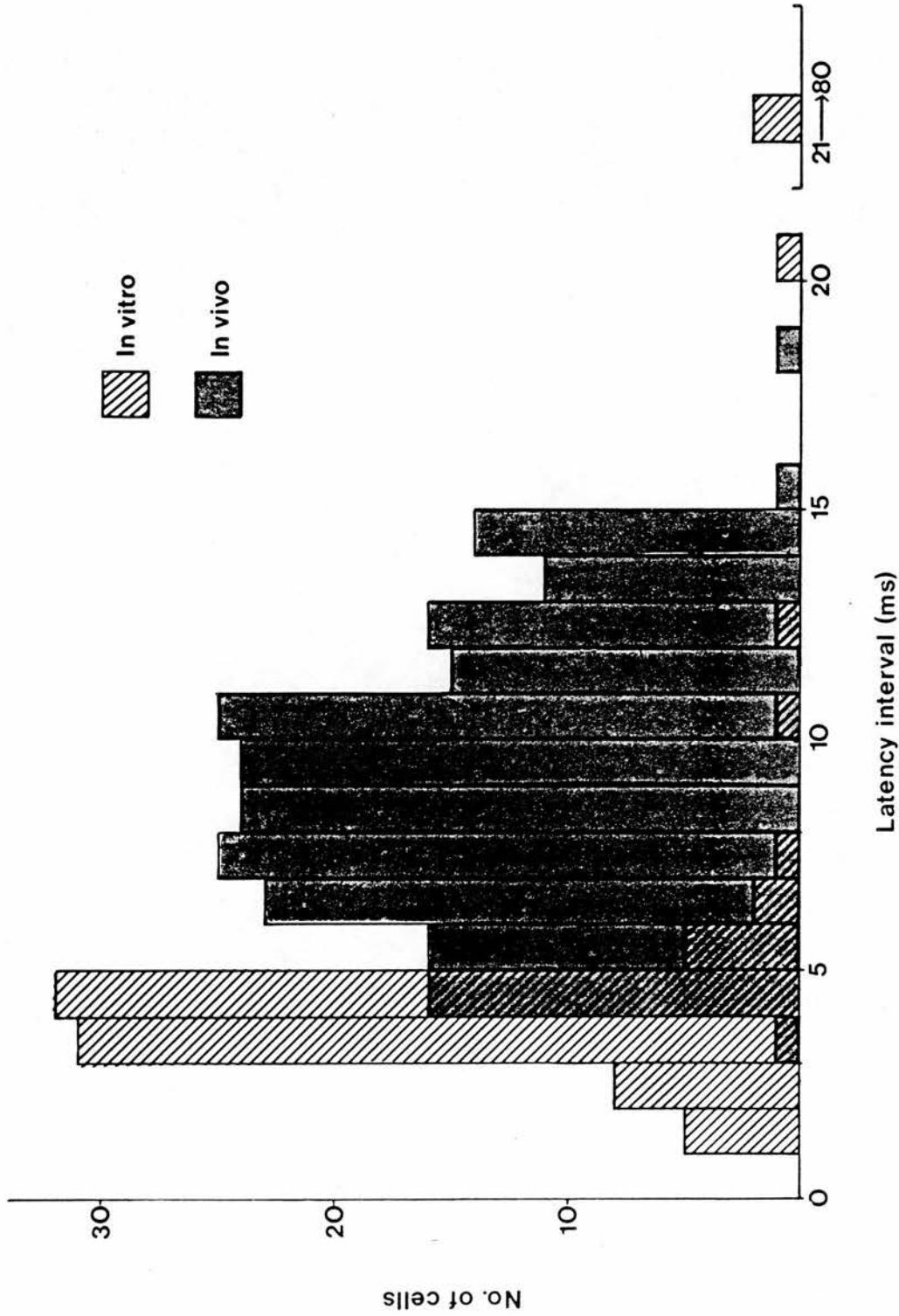


FIGURE 34: Latency interval histogram.  
Response latencies recorded 'in vitro' are compared to  
those previously recorded 'in vivo' (from Brown, 1981).

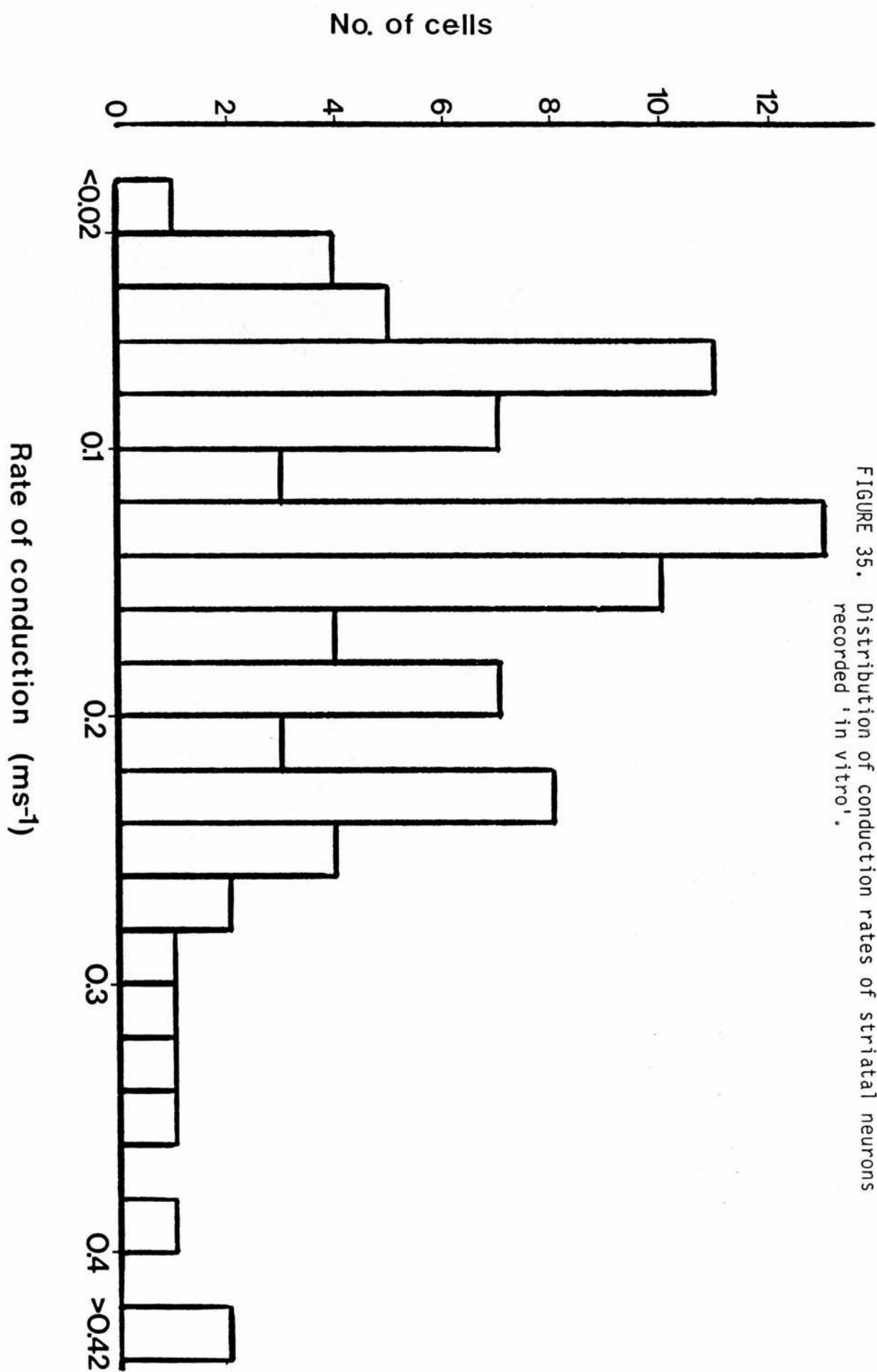


FIGURE 35. Distribution of conduction rates of striatal neurons recorded 'in vitro'.

Rates of conduction for these neurons were calculated by measuring the distance between the stimulating electrode and the position of the recording electrode within the striatum, and dividing this by the latency of the response. The mean rate of conduction for all cortically driven neurons was calculated to be  $0.15 \pm 0.09\text{ms}^{-1}$  ( $n = 89$ ). A histogram of conduction rates is shown in Figure 35.

It has been found that intracellularly recorded striatal neurons show an initial excitation followed by a prolonged inhibitory phase after cortical stimulation (Herrling, 1984; Marco et al, 1973; Morris et al, 1979). A procedure for testing this inhibitory phase of the cortical response was outlined on page 130. If a wide enough interval was left between two pulses a second response was always observed which was usually of the same amplitude or slightly smaller than the initial response. As the interval between pulses was decreased the second response was continually observed until such a point where the interval between the pulses was too short to allow a second response to occur. The interval at which the second response was just still visible was the interval which was used in the calculation of a mean value for the inhibitory phase ( $14.0 \pm 5.06$  ms,  $n = 6$ ) and an example of this is shown in Figure 36.

It was observed during the course of this study that some recording tracks yield up to 6 or 7 activated cells while in others in the same preparation, only one or two neurons or sometimes more were activated by cortical stimulation (see Figure 37). This led to the suggestion that the preparations were displaying a "patchiness" of cortical inputs (c.f. Gerfen, 1984), such that some areas were relatively quiet in terms of their cortically evoked activity whereas other were not.

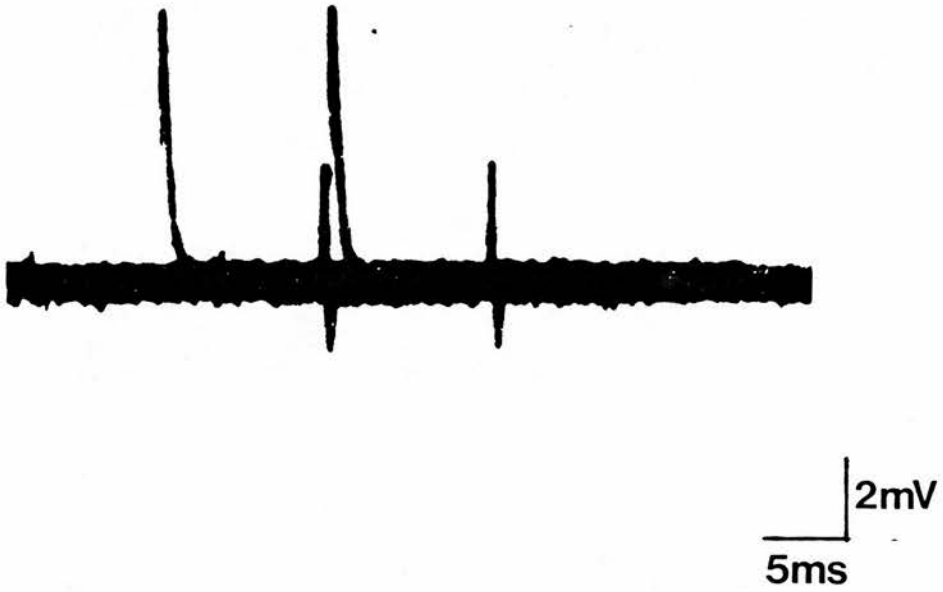


FIGURE 36. Paired-pulse stimulation of a striatal neuron.  
The interval between stimuli is 10ms.  
With shorter intervals the second response disappeared.

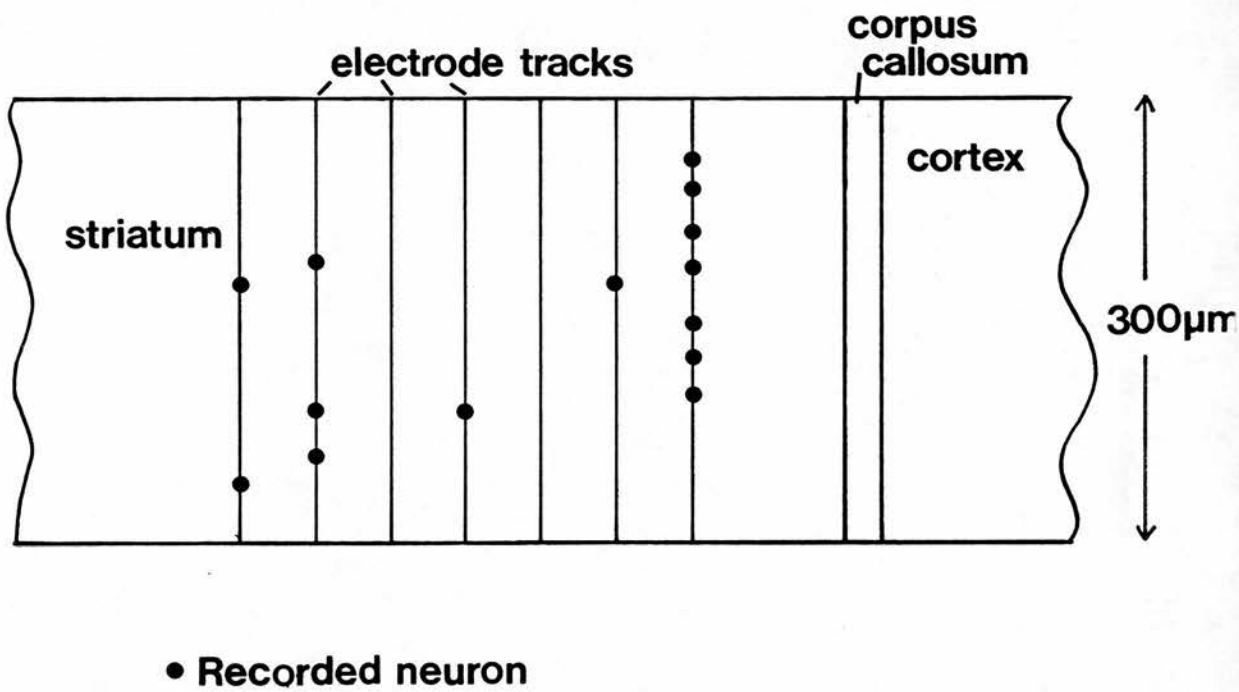


FIGURE 37: Diagrammatical representation of a typical recording experiment. This represents a vertical section taken through a 300  $\mu\text{m}$  thick striatal slice. Each vertical line within the striatum represents an individual electrode track, which are approximately 50  $\mu\text{m}$  apart. Recorded neurons are represented by filled circles.

## DISCUSSION

The present results provide evidence to suggest that corticostriatal inputs are maintained in the parasagittally cut slices. By cutting at precisely the required angle the corticostriatal fibre bundles traversing the striatum are left intact. The previous chapter demonstrated that these fascicles were densely labelled after injection of PhAL into the cortex, so it can be assumed that the bulk of corticostriatal fibres remain intact in the preparation used in these experiments.

The difficulty in retaining this pathway 'in vitro' is in direct contrast to the situation in the hippocampus which has a well defined morphological organisation such that distinct cellular or dendritic layers are easily visualised and distinct fibre bundles are present for stimulation. This has made the hippocampus directly amenable to physiological examination so that it has received a great deal of attention in terms of 'in vitro' slice experiments (e.g. Langmoen and Anderson, 1981; Asaf et al., 1981; Schwartzkroin, 1975; Dingledine et al., 1980).

Considering the importance of the corticostriatal pathway in the modulation of striatal function, it is hard to understand why a slice preparation of this pathway has not become a well-established research tool. Only one other 'in vitro' study has apparently attempted to utilise a preparation similar to that used here (Miller, 1981), but also examined the responses to local stimulation as well as to corticostriatal stimulation, local stimulation being the method of choice in almost all 'in vitro' experiments on the striatum.

The difficulty in using a preparation of the type described here comes with the uncertainty in determining whether or not the striatal neurons have been stimulated directly rather than via afferent inputs. This is a distinct possibility because of the likelihood of current spread over the surface of the slice. Several factors are likely to control this spread, namely the size of the stimulating electrode, stimulating currents used, the amount of fluid covering the surface of the slice, and the distance between the stimulating and recording electrodes. Ranck (1975) postulated that currents up to  $500\mu\text{A}$  could excite axons up to 1mm distant from the stimulation site 'in vitro'. While many of the recordings in this experiment were made at distances less than 1mm from the stimulation site, it is unlikely that many of them have been stimulated directly because of the following reasons:

1. Most response latencies were greater than 2ms, which suggested that these would not be direct responses. Those neurons recorded close to the stimulating electrode (i.e. less than 0.2mm away) tended to have response latencies of less than 2ms.
2. Only "strips" of grey matter lying directly below the stimulating electrode could be activated. If the recording electrode was moved to a parallel "strip", no recordings were obtained, which suggested that lateral current spread was minimal.
3. Response latencies were very similar to those found 'in vivo' (Brown, 1981; Vandermaelen and Kitai, 1981).

Results of extracellular recordings obtained in this preparation are similar in many respects to corresponding records obtained both 'in vivo' and 'in vitro'. For instance, of 91 cortically driven

neurons in the slices, 15.4% showed a pattern of spontaneous activity similar to striatal neurons 'in vivo' (Brown, 1981; Schultz and Ungerstedt, 1978). Brown (1981) found that of 218 cells similarly recorded 'in vivo', 16% had such activity (see table 4) while Marco et al. (1973) found that 35% of their recorded neurons were spontaneous. A similar experiment carried out 'in vitro' (Miller, 1981) demonstrated that 22% of the neurons were spontaneously active, while Kitai and Kita (1984) recorded no spontaneous activity whatsoever.

The typical response to cortical stimulation 'in vivo' and 'in vitro' is a short latency excitation which can be seen to result from a sharply rising depolarising synaptic potential in intracellular records. Figure 34 summarises the response latencies from 'in vitro' recordings obtained here and previous 'in vivo' recordings from this lab (Brown, 1981). This shows that 'in vitro' a larger proportion of short latency responses are present which tends to reflect the ease with which recording sites can approach the stimulation site. However, at the smaller separations, as mentioned earlier, direct responses may also distort the latency distribution. The lack of longer latency responses in the 'in vitro' slice preparation can be attributed to the isolation of the striatum from most of its inputs, such that longer latency responses to monosynaptic and polysynaptic inputs would be abolished. It has been shown that a proportion of striatal responses may be mediated via the cortico-thalamostriatal pathway 'in vivo' (Cherubini et al., 1979) which are of much longer latency than those described here. It is likely therefore that removal of the striatum from most of its inputs might help to explain why response latencies, on the whole,



tend to be shorter than those obtained 'in vivo'. The response latencies observed here are comparable with those obtained by Miller (1981) although differences in the type of medium used, preparation of the slice (in terms of orientation) and the temperature of the bathing medium may explain any slight differences observed.

Like other 'in vitro' experiments this study demonstrated the presence of an inhibitory phase of the response to cortical stimulation. This was discovered in two ways:

1. Spontaneously active neurons which were activated by cortical stimulation were found to respond with an initial excitation followed by a period of inhibition generally lasting 15-90ms.
2. Paired pulse stimulation, which is a different independent measure of the inhibitory period, gave rise to a mean interstimulus interval of  $14.0 \pm 5.06\text{ms}$ .

Thus the inhibition of spike generation is shorter after paired-pulse stimulation than that observed with spontaneous firing. The values obtained for the paired-pulse stimulation experiments are in keeping with the hypothesis that a short latency inhibitory mechanism exists within the striatum, a phenomenon which has been demonstrated in other slice experiments (Lighthall et al., 1981; Lighthall and Kitai, 1983), as well as 'in vivo' (Herrling, 1984; Wilson et al, 1983b). On the other hand, those values obtained for the inhibitory periods of spontaneously active neurons responding to cortical stimulation tended to be longer and more in keeping with the proposal of a long-lasting inhibitory phase (albeit shorter than most 'in vivo' values).

Two explanations might account for these observations. Firstly, it is possible that the method used here overcomes the cortical

disfacilitation proposed by Wilson et al (1983) so that neurons are made to fire earlier on. Secondly, the cortically-evoked EPSP might be larger than whatever excitatory process is responsible for spontaneous activity so the firing threshold is reached more quickly, thus decreasing the inhibitory phase of the response. Either way, the presence of an extrinsic inhibitory mechanism such as that of cortical disfacilitation (Wilson et al., 1983) cannot be ruled out on the strength of the present results; while it seems unlikely that such a mechanism would remain entirely intact in a slice, it is possible that it may be present in some form.

The rates of conduction of corticostriatal fibres were estimated for the present results and also for the previous 'in vivo' results of Brown (1981). Corticostriatal fibres have been shown to have thin, poorly myelinated axons upon entering the striatum (Webster 1961) and are usually considered to have conduction velocities in the range of  $0.5\text{--}1\text{msec}^{-1}$  (Waxman and Bennett, 1972) which was somewhat faster than the estimate obtained for 'in vitro' recorded neurons ( $0.15\pm 0.09\text{msec}^{-1}$ ). However, other 'in vivo' studies have calculated rates of conduction to be similar to those of Waxman and Bennett (Sedgewick and Williams, 1967; Vandermaelen and Kitai, 1980). The estimation of conduction rates in both 'in vivo' and 'in vitro' studies are subject to a great deal of error. This is mainly because distances between stimulating and recording electrodes cannot be measured accurately; 'in vivo' the distance cannot be worked out directly but has to be obtained from the stereotaxic coordinates used to place the electrodes, while 'in vitro' the sources of error come from determining the exact position of the electrode tip and also from the measurement of the very small

distances involved. The fact that the corticostriatal pathway is not necessarily straight also has to be considered such that fibres may be somewhat convoluted in their passage into the striatum. In this study, it was the direct distance between stimulating and recording electrodes which was estimated for the 'in vitro' results and those from the earlier 'in vivo' study, and which might account for the estimates of conduction rate being rather low in comparison to other studies.

The present findings also lend some support to Miller's (1981) observations which tend to suggest that the striatum is organised into distinct functional units. However, he implied that responding neurons are organised into columns traversing the striatal slice in a plane parallel to the corticospinal fibre bundles, an assumption made after the observation that recording in parallel "strips" did not obtain any responses. While the present results can neither refute nor confirm the presence of columns as such, it does seem possible that there is some heterogeneity in the distribution of striatal neurons responding to stimulation of one particular cortical site (although this "heterogeneity" might equally reflect the severing of certain inputs during the slicing process). Neither the present study nor that of Miller attempted to observe any convergence of inputs from separate cortical areas or any widespread input from any one particular cortical site. Until this is carried out in a slice preparation then it cannot be determined whether the inputs lie in columns or in clusters.

Anatomical investigations have described, as outlined in the previous chapter, various cytoarchitectonic arrangements within the striatum which might form a structural basis for such organisation

within this nucleus. Ring-like clusters of neurons and dendrites have been detected in striatal sections cut perpendicular to the fibre bundles (Mensah, 1977; Gerfen, 1984; Donoghue and Herkenham, 1986). On the other hand, parallel sections show columns of neurons with extensive dendritic intertwining (Chronister et al., 1976). Autoradiographic studies have also indicated that terminal distribution of thalamic (Kalil, 1978) and cortical (Goldman and Nauta, 1977; Selemon and Goldman-Rakic, 1985) afferent fibres in the striatum are organised into patch-like regions which, if the striatum is sectioned in a particular way, also give the impression of columns.

As the present study only gives a preliminary insight into this functional arrangement it is obvious that more work has to be carried out on this subject. For instance, it has yet to be proved conclusively that this particular preparation does indeed maintain intact corticostriatal fibres so that the results of local stimulation do not contaminate the present results. This could be done in several ways: by pharmacological studies using excitatory amino acids and their antagonists; or by placing a small knife cut between cortex and striatum in the slice (see Langmoen and Andersen, 1981) to interrupt any existing cortical inputs. Further work to investigate the possibility of a functional arrangement of cortical inputs would require the intracellular recording of striatal neurons responding to cortical stimulation. By filling these neurons with HRP and subsequently staining sections taken from the slice with antibody to CaBP (Gerfen et al, 1985) it could be determined whether or not the responding neurons were situated within neurochemically distinct compartments within the striatum, as has been previously

described anatomically for corticostriatal inputs (Gerfen 1984; Ragsdale and Graybiel, 1981; Donoghue and Herkenham, 1986).

In conclusion, certain properties of the corticostriatal system appear to have been preserved in these slices. This therefore allows for the possibility to directly test the pharmacological theories of how corticostriatal and local striatal transmission occurs and to study in greater depth the apparent functional organisation of the striatum.

#### SUMMARY

1. Corticostriatal inputs appear to be maintained in an 'in vitro' slice preparation.
2. The majority of neurons were silent and only responded to stimulation of the cortex with a mean latency of  $4.35 \pm 3.35$ ms
3. Properties of these neurons were compared to those obtained 'in vivo' and were found to be similar.
4. A short inhibitory phase of the cortical response was demonstrated.
5. Evidence to suggest that the cortical input to striatum occurs in a patchy manner was also presented.

## CHAPTER 4

### The Electrophysiological Demonstration of Host Cortical Inputs to Striatal Grafts

## INTRODUCTION

In the last 10-15 years much attention has been focused on the transplantation of nervous tissue as a means of repairing damaged neuronal circuits, (e.g. Bjorklund and Stenevi, 1984; Freund et al., 1985; Mahalik et al., 1985; Olson, 1985, Dunnett and Bjorklund, 1987; Bjorklund et al., 1987) to study neuronal development, and functional organisation within the mammalian central nervous system (CNS). A survey of the relevant literature however, shows that experiments of this nature have been carried out frequently since the end of the last century.

The first report of such an experiment was made by Thompson in 1890, in which he transplanted cat cortical tissue into dog brain. The results of this study were confusing and inconclusive.

Other early experiments were carried out more successfully by Fossman (1898; 1900) who showed that peripheral nerve implants would attract ingrowing CNS neurons while other tissue would not. He defined this ability as a "neurotropic action". Tello (1911) came to essentially the same conclusion by implanting pieces of sciatic nerve into rabbit brain, suggesting that chemical factors released from the implant could be responsible for directing damaged axons towards the implant.

Subsequent experiments carried out by other researchers also met with reasonable success. In particular those of Ranson (1903; 1914), Dunn (1917) and le Gros Clark (1940), in which embryonic neocortex was grafted into the cortex of 6-week old rabbits, resulted in particularly good survival and differentiation of graft neurons.

These results, however, were very much more the exception than the rule; in general terms, results obtained from this type of experiment were poor. The studies of Saltykow (1905) and those of Del Conte (1907) (which was the first attempt to place embryonic tissue grafts into mammalian brains) were, like the Thompson study, confusing and difficult to interpret. Other negative results came from the experiments of various researchers, e.g. Altobelli et al (1914), Willis (1935), Glees (1955) and Frotscher et al (1970). Even le Gros Clark, after his moderately successful experiments published in 1940, remained sceptical about the beneficial effects of transplantation. In 1942 he carried out a study on the influence of spinal ganglia and peripheral nerve fibres on regeneration in the brain. Essentially, his results confirmed the findings of Tello (1911), but his apparent scepticism brought him to interpret his data in a negative manner.

Although the vast majority of transplant studies carried out during the 80 years following Thompson's experiment (1890) were failures, there were some successes. Indeed it is surprising, in view of these occasional successes, that this field was not more actively researched. This can probably be explained by the fact that the prevailing opinion up until the 1960s was that first argued by Cajal (1928) after a series of experiments on regeneration in the spinal cord of young animals. He referred to the slight regeneration observed as "abortive regeneration" in that sprouting did occur from cut axons, but that the process soon terminated and the sprouts degenerated. His conclusion was that regeneration and growth did not take place in the adult mammalian brain. This view was confirmed to a great extent by various researchers including



le Gros Clark (1942), Brown and McCouch (1947), Windle (1956) and Guth and Windle (1970). Consequently, reports of viable neural transplantation such as that of le Gros Clark (1940) were received with a great deal of scepticism and were largely ignored.

Later experiments, however, did provide further evidence to suggest that more persistent and functional regeneration could take place, effectively challenging the widely accepted views of Cajal (1928). For example, Raismann (1969) gave the first electron microscopic demonstration of collateral sprouting of axon terminals in partially deafferented septum. This prompted the reconsideration of the regenerative capacity of neurons and led to a renewed interest by various groups who employed neuronal tissue grafts to observe and manipulate groups of neurons in development and regeneration of axonal connections in the brain (Das and Altman, 1971; 1972; Bjorklund and Stenevi, 1971; Lund and Hauscka, 1976) and in the anterior chamber of the eye (Olson and Malmfors 1970).

A study carried out by Fuxe et al (1969) in which they examined the distribution of monoamines in the mammalian CNS by histochemical methods after lesioning, gave clear indications of significant positive regeneration of these neurons. This study prompted Bjorklund and Stenevi (1971) to examine the ability of cerebral monoaminergic neurons to grow into transplants of peripheral tissue in albino rats. Both DA- and noradrenaline (NA)- containing fibres which had been surgically lesioned, were observed to abundantly invade transplants of iris and mitral valve tissue. The importance of transplant position, types of neurons involved and the nature of the transplanted tissue in determining the magnitude and distribution pattern of the regenerating fibres was also demonstrated.

This report, and a later extension (Stenevi, Bjorklund and Svendgaard, 1976) in which the characterisation of the conditions for reliable graft survival and growth were formulated, played a key role in sustaining the renewed interest in transplantation. They showed that there were three factors which played a critical role in the ability of grafts to survive:

- (i) Viability of donor tissue is greater when obtained from immature (particularly embryonic) donors.
- (ii) The selection of a transplant site within the host brain which is highly vascularised in order to provide support and rapid incorporation of newly grafted tissue into the host. A subsequently developed measure is the construction of a vascular bridge (Stenevi et al., 1976); or the transplantation procedure can take the form of a two-stage process (see Bjorklund and Stenevi, 1979; Stenevi, Bjorklund and Dunnett, 1980).
- (iii) Immunological factors are less important to consider in the brain due to it being an "immunologically privileged" site (Medawar, 1948). The use of aseptic conditions however, is a very important factor to consider in achieving successful transplants.

Thus, by the mid-1970s, the few early findings that CNS neurons do undergo regeneration had been greatly substantiated and it is now known that many parts of the neuraxis can be transplanted not only into the CNS of adult animals, but also developing (Das, 1974; Das et al., 1980; Sunde and Zimmer, 1983) and aged recipients (Gage et al., 1983).

Transplants of a wide variety of brain areas including the hippocampus (Kromer, Bjorklund and Stenevi, 1981a, 1981b; Olson et al., 1977), septum (Bjorklund, Kromer and Stenevi, 1979; Bjorklund and Stenevi, 1977; Dunnett et al., 1982; Lewis et al., 1980), cerebellum (Das, 1973; Das and Altman, 1971; 1972; Hoffer et al., 1974; Wallace and Das, 1982), spinal cord and ganglia (Rosenstein and Brightman, 1979; Thuline and Bunge, 1972), hypothalamus (Arendash and Gorski, 1982; Gash and Sladek, 1980; Gash, Sladek and Sladek, 1980; Krieger et al., 1982; Stenevi et al., 1980), cortex (Jaeger and Lund, 1979; Labbe et al., 1983; le Gros Clark, 1940; Fonseca et al., 1988; McLoon and Lund, 1983), substantia nigra (Bjorklund et al., 1980; Bjorklund, Schmidt and Stenevi, 1980; Bjorklund and Stenevi, 1979; Dunnett et al., 1981a, 1981b, 1981c; Fray et al., 1983; Freed et al., 1980; Gage et al., 1983; Perlow, 1980; Perlow et al., 1979; Stenevi, Bjorklund and Dunnett, 1980; Bolam et al., 1987) and striatum (Deckel et al., 1983, 1986a, 1986b; Isacson et al., 1984; Schmidt, Bjorklund and Stenevi, 1981; Isacson et al., 1985; 1986; 1987a; 1987b; Bjorklund et al., 1987; Dunnett and Bjorklund, 1987; Pritzel et al., 1986) have all been shown to survive after transplantation and to reverse behavioural, anatomical and neurochemical deficits resulting from prior lesions in the host.

Of all brain areas, the striatum is probably the most extensively examined, in terms of behavioural, morphological and functional studies of grafted neurons. In particular the nigral input to striatum has been frequently utilised in these studies and DA-rich grafts placed within or near the striatum have been found to compensate for behavioural deficits induced by neurotoxic lesions of

the nigrostriatal DA pathway in adult rats (see above for references).

In 1979, two groups independently used the nigrostriatal model to provide the first demonstration that neural grafts placed in close apposition to the striatum can compensate for and reduce behavioural effects induced by brain damage in adult mammals. Perlow et al (1979) transplanted DA-rich nigral grafts into the ventricles of 6-hydroxydopamine(6-OHDA)-lesioned rats. This led to an approximately 40% decrease in the apomorphine-induced turning asymmetry. Bjorklund and Stenevi (1979) on the other hand, placed similar grafts in a cortical cavity overlying the dorsal striatum and obtained complete compensation of amphetamine-induced turning.

These findings raised the possibility that elements within the striatal neuronal circuitry, other than nigrostriatal DA neurons, might be amenable to replacement by grafted neurons after intrinsic damage to the striatum. Of particular interest in this instance was the discovery that injections of the glutamate analogues kainic acid (KA), (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Schwarcz and Coyle, 1977) or ibotenic acid (IA), (Schwarcz et al., 1979) produced an extensive loss of intrinsic neurons within the striatum whilst sparing fibres of passage (KA being the much more extensively damaging of the two). Concurrent with this neuronal loss were alterations in behaviour, brain neurochemistry and glucose utilisation which were akin to those observed in Huntington's Chorea (Mason and Fibiger, 1978; 1979; Bird, 1979; Kelly et al., 1982; Dunnett and Iversen, 1980; 1981).

Several subsequent studies have explored the potential of grafted fetal striatal neurons implanted in IA-lesioned striatum to

compensate for the structural (Deckel et al., 1983, 1986; Isacson et al., 1984; 1985; 1986; 1987a; 1987b; Clarke et al., 1988; McAllister et al., 1985; Schmidt, Bjorklund and Stenevi, 1981), neurochemical (Isacson et al., 1984; 1985; Schmidt, Bjorklund and Stenevi, 1981; Sirinathsinghji et al., 1988) and behavioural (Deckel et al., 1983; 1986; 1987; Dunnett and Iverson, 1981; Isacson et al., 1984; 1986; Dunnett et al., 1988) deficits observed.

All of these studies have demonstrated that developing striatal neurons taken from 14-15 day old fetuses survive grafting as either a neuronal cell suspension (see Bjorklund et al., 1980) or as a "slab" transplant (see Das, 1974) in KA or IA lesioned striatum. The grafts have been found to proliferate and produce cell aggregates within the area of the lesion. They appear to restore GAD and ChAT levels in the lesioned striatum, while glucose utilisation in several striatal target areas has been normalised after implantation (Isacson et al., 1984). A partial reversal of the locomotor hyperactivity and 'cognitive' impairments seen in lesioned rats has also been observed after transplantation of striatal tissue.

In general terms, the internal organisation of most types of graft have been well characterised anatomically, neurochemically and behaviourally, with striatal grafts being no exception as outlined above. However, unlike in other systems, no reports exist to indicate whether the anatomical connections of striatal grafts are functional at the electrophysiological level.

This approach has provided important information on the functional connections of DA grafts in the striatum (Stromberg et al., 1985; Arbuthnott, Dunnett and MacLeod, 1985; Wuerthele et al., 1981). Arbuthnott, Dunnett and MacLeod (1985) used antidromic

stimulation to identify those neurons projecting from nigral grafts to innervate host striatum. In rats in which the amphetamine-induced rotation response was compensated, both DA-like and non DA-like units were identified whereas in uncompensated rats, all units had spontaneous waveforms and conduction velocities characteristic of the latter type of unit. Additionally, electrophysiological evidence of afferent input to grafts by orthodromic stimulation of locus coeruleus, raphe nucleus and neocortex was obtained although these inputs have not yet been confirmed by anatomical tracing techniques.

Similar findings were obtained by Trulson and Hosseini (1987) in freely moving cats where it was found that the transplanted dopamine neurons discharged at a faster rate and never displayed the characteristic decremental burst pattern seen in intact animals. Similarly, electrophysiological recording from cholinergic and noradrenergic grafts in the hippocampus (Bjorklund, Segal and Stenevi, 1979; Low et al., 1982; Segal et al., 1985) suggest that neurons grafted into the hippocampus form physiologically viable connections.

Although all of these electrophysiological recordings have been made 'in vivo', identification of the exact position of the graft and stability of the preparation can prove difficult. Both of these problems can be overcome by the use of 'in vitro' slice preparations, an approach used successfully by Segal et al (1985) to identify the extent and pharmacological specificity of cholinergic fibre outgrowth from septal grafts in hippocampal slices.

The aim of this particular study was therefore to determine, using 'in vitro' electrophysiological techniques, whether striatal

grafts receive functional inputs specifically from the prefrontal cortex, the major afferent connection of the striatum. Responses to these inputs were also compared to those seen in slices of normal striatum.

## METHODS

### Animals

The experiments employed 16 young adult female Sprague-Dawley rats. An additional four animals were used as controls.

### Striatal lesions

16 rats were anaesthetised with 3.0ml/kg equithesin (81ml nembutal, 21.25g Chloral hydrate, 10.63g magnesium sulphate, 198ml propylene glycol, 50ml alcohol, plus water to 500ml) and were mounted in a stereotaxic frame (Kopf Instruments). The IA used to produce the lesions was dissolved to a concentration of 0.05M in phosphate buffer at pH7.4. The pH was readjusted to between 6 and 7 by the addition of sodium hydroxide (3-4 $\mu$ l of a 1M solution per 100 $\mu$ l of the IA solution). The IA was stored in ice out of direct light during surgery.

Each rat had part of the skull removed anterolateral to Bregma using a fine drill. A fine needle (30 gauge) mounted on a Hamilton syringe was lowered unilaterally at two stereotaxically defined sites (A = 1.6mm, L = 1.5mm, V = 4.5mm and A = 0.0mm, L = 3.0mm, V = 4.5mm). At each site 0.5 $\mu$ l of IA was injected over 10 minutes before the needle was slowly retracted. Finally, the scalp wound was sutured and the animals placed in recovery cages where their progress was monitored. After 1-2 hours they were returned to their home cages and maintained on a 12:12 hour light:dark cycle with unlimited access to food and water.



### Transplantation

A summary diagram of the transplantation procedure is outlined in Figure 38.

Approximately two weeks after the IA lesion the rats received striatal implants according to the cell suspension technique (Bjorklund et al., 1983) using tissue from the striatal eminence dissected from 13mm long (14-15 day gestational age) embryos of the same rat strain.

Fetuses were sequentially removed from the uterine horns of an anaesthetised pregnant rat and the brains were dissected out using microsurgery forceps and scissors under a dissecting microscope. They were then placed in a petri dish containing 0.6% glucose-saline and cut open through the cortex. The striatal eminences were carefully cut out of both hemispheres and stored in the medium (1 and 2, figure 38).

Striata from one litter were incubated for 20 minutes at 37°C in 0.1% trypsin (Sigma, Type II) dissolved in the glucose-saline medium(3). The trypsin was removed by repeated washing of the tissue (changing the medium 5 or 6 times, 350μl per time). To produce a dissociated cell suspension, the pieces were sucked through the opening of a fire-polished Pasteur pipette until a milky suspension was obtained(4). The final suspension contained approximately two striatal eminences per 10μl of medium, its viability being checked microscopically(5).

Using the same procedure as described above 3μl deposits of cell suspension were implanted into the striatum of all lesioned hosts halfway between the two lesion injection sites (A = 0.8mm, L = 3.00mm, V = 4.5mm), over a period of 10-12 minutes(6). After

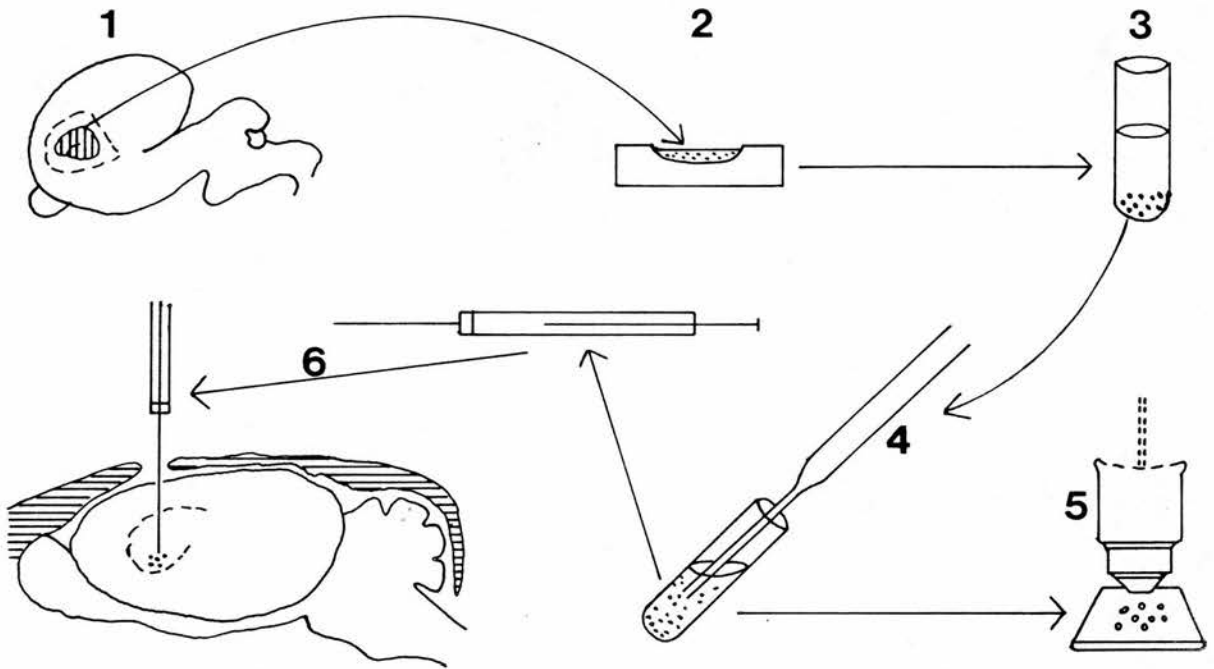


FIGURE 38. Diagrammatic representation of the principle steps involved in the neural grafting procedure. This method is outlined in greater detail on pages 161 & 163.

Redrawn from Isacson (1987)

injection the needle was retracted very slowly.

Half of the host animals received embryonic graft tissue labelled with rhodamine-fluorescent latex microspheres (0.02–0.2 $\mu$ M, Tracer Technologies Inc.) at a concentration of one embryonic tissue piece per 5 $\mu$ l. No such label was applied to the grafts in the remaining hosts. This was carried out to enable the boundaries of the grafts to be located with more certainty – an important factor in the consideration of microelectrode location (see Electrical recordings, below).

#### Electrophysiological recording experiments

Six months after transplantation the rats were killed by decapitation. The procedures carried out were as described in Chapter 3, Methods section.

#### Electrical Recordings

Striatal neurons from control animals and graft neurons from transplanted animals were recorded extracellularly with glass electrodes (Clark Electromedical Instruments, GC 150F-10) filled with either 2% Pontamine Sky Blue in 0.5M sodium acetate (DC resistance 40–80M Ohms) or occasionally 3M potassium chloride (DC resistance 30–50M Ohms) filled electrodes were used as an alternative. Pontamine, however, allowed for much easier visualisation of the electrode tips. The rhodamine bead-containing grafts were viewed under ultraviolet illumination to allow for accurate placement of electrodes within the grafted tissue. In other cases where rhodamine beads were not present, the boundary of the grafts were usually very well-defined and could be detected

quite easily with a dissecting microscope. In control slices, recording was confined to the areas of grey matter between corticostriatal fibre bundles. The distance between the stimulating electrode and the position of the recording electrode within the striatum or graft was noted in all cases (see Figure 39).

#### Stimulating electrodes

Stimuli (0.5-100V, 0.1ms pulse width, 1Hz) were applied to a stainless steel wire placed on the surface of the slice at the edge of the corpus callosum nearest the cortex. In all cases the recording electrode was at least 1.0mm from the stimulating site. Control experiments involving intracellular recording from striatal neurons demonstrated that direct stimulation by current spread could only be detected with stimulus intensities greater than twice as high as any employed in this series of experiments (see Chapter 3). Additionally, direct antidromic stimulation results in response latencies less than 1msec, whereas all latencies observed here were more than 2msec.

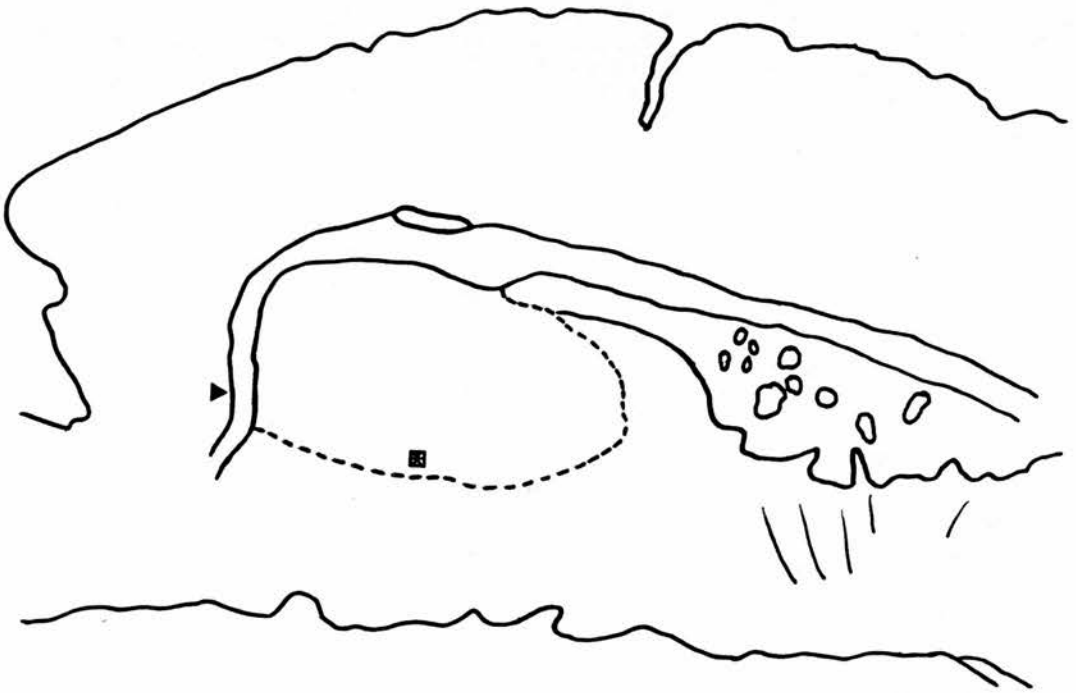
Neuronal activity was amplified, filtered (-3dB at 500 Hz-10KHz) and displayed on an oscilloscope (Tektronix).

#### Paired-pulse stimulation

This procedure was carried out as detailed in Chapter 3 (page 130).

#### Analysis of results

Mann Whitney U-tests were used to determine if there were any



- ▴ Stimulating electrode
- Recording electrode

FIGURE 39. Diagram showing relative positions of recording and stimulating electrodes within a slice of grafted striatum. The boundary of the graft is represented by the dotted line.

significant differences between the results obtained for the two populations of neurons from control and grafted animals.

### Histology

Blocks of tissue from which slices had been cut were dropped into phosphate-buffered 4% paraformaldehyde and 0.05% glutaraldehyde and left for several hours in a refrigerator. In a similar manner, slices from which recordings had been made, were carefully removed from the recording chamber at the end of an experiment and fixed in the same solution for approximately 30 minutes.

After fixation the tissue was immersed in 20% sucrose until sectioning (> 24 hours). Both blocks and slices were cut on a freezing microtome into 50 $\mu$ m thick sections. Sections were either stained using standard AChE procedures or using TH immunohistochemistry. These were left in phosphate-buffered saline (PBS) + Triton + azide until required. Some sections from rhodamine-labelled grafts were fixed, coverslipped and viewed under a fluorescent microscope to examine the extent of growth and distribution of the grafts.

### AChE staining procedure

This was carried out according to Koelle's thiocholine method (for modification see Holmstedt, 1957) using prometazine ( $10^{-4}$ M) as an inhibitor of non-specific esterases. The sections were very lightly counterstained with haematoxylin and eosin.

### Immunohistochemistry

TH-immunohistochemistry was carried out using a modification of a method used by Gerfen and Sawchenko (1984) for PhAL staining. The sections were incubated in 20% goat serum for two hours, rinsed and then incubated in the primary antibody (rabbit anti-rat TH) overnight at 4°C. They were then given several washes with PBS after which they were incubated in a 1:200 dilution of biotin secondary antibody (Vector) for approximately one hour, rinsed again and incubated for 30 minutes with a 1:100 dilution of A + B (Vectastain) in PBS containing no Triton or azide. Further washes were carried out with PBS followed by several washes in 50mM Tris buffer (pH 7.4). The sections were then incubated in a solution of 5mg of 3,3'-diaminobenzidine (DAB)(Sigma) in 50mM tris buffer for ten minutes and for a further ten minutes after the addition of DAB + 3% Hydrogen peroxide ( $H_2O_2$ ). These procedures were carried out at room temperature unless otherwise stated.

The sections were then rinsed in PBS, mounted carefully on "subbed" slides, left to dry at room temperature and coverslipped.

## RESULTS

### Appearance of grafts

Determination of the graft position within host striatum was usually very easy when slices were viewed under the dissecting microscope. In the case of non-rhodamine labelled grafts the head of the host striatum occasionally contained graft tissue which clearly extended over approximately one-third to one-half of the head area in the rostrocaudal and ventromedial axes (Figure 40). The boundaries of these grafts were very clearly defined so that no difficulty occurred in ensuring that the recording electrode was positioned within graft tissue rather than host tissue. In the case of bead-labelled grafts however, the edges of the grafts were not so clearly defined and indeed the grafts themselves tended on the whole to be smaller in diameter than the non-labelled grafts (Arbuthnott and Dunnett, personal communication) with what appeared to be very white scar tissue marking out a diffuse boundary round the edges. This did not seem to affect the ability to record from these grafts as many recordings were obtained from preparations of this type. However, the fact that they appeared to be a lot smaller than non-beaded grafts restricted the number of recording tracks which could be attempted in each individual experiment.

When viewed under a fluorescent microscope it was observed that the rhodamine-labelled grafts did extend over a fairly large area (Figure 41), as some labelled neurons could be seen to have migrated somewhat from the injection site, which suggests that the grafts had grown to a considerable extent.



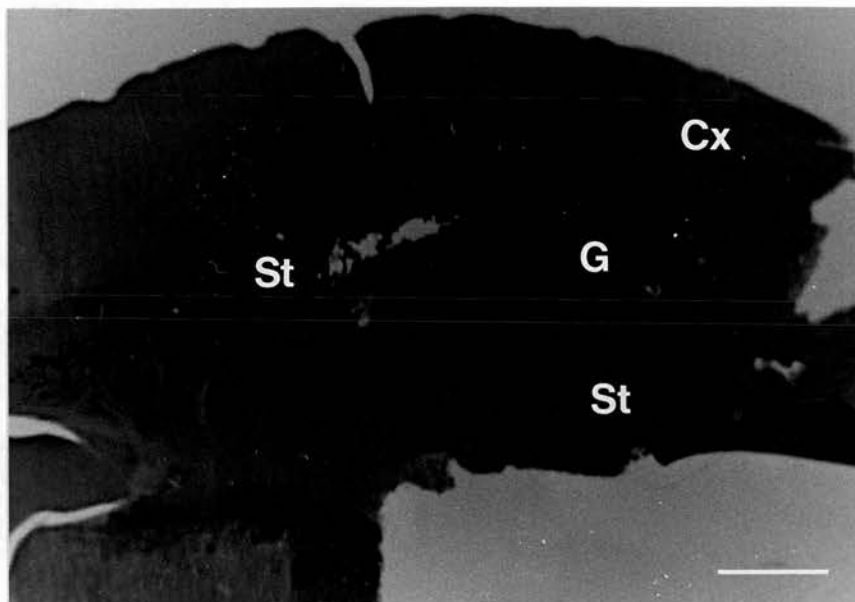


FIGURE 40. Photograph of a 50µm AChE-stained section. This section was taken from a tissue slice after a recording experiment. Scale bar = 1mm

Abbreviations:

cx cortex  
G graft  
St striatum

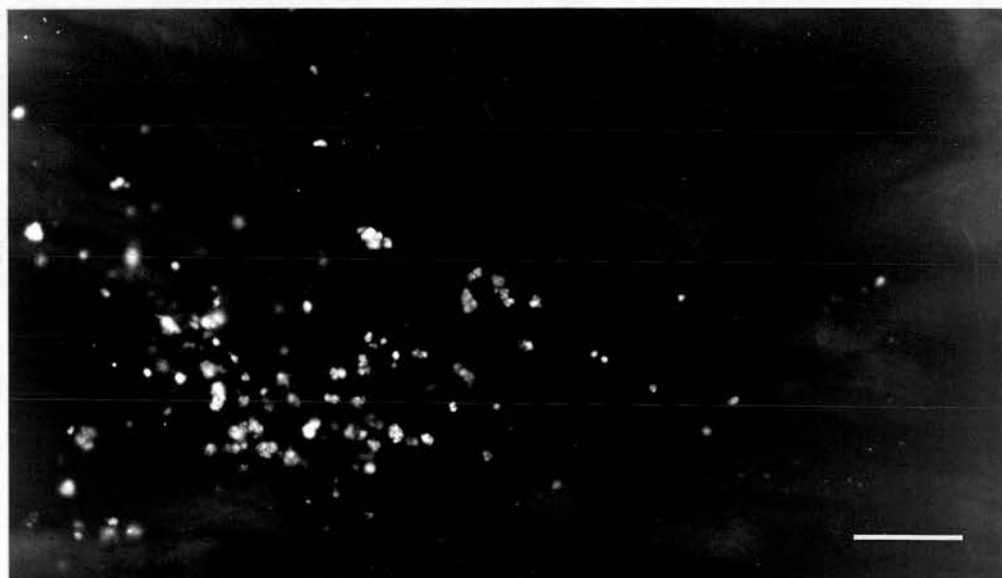


FIGURE 41. High-power photograph of a striatal graft.

The section (50µm) was taken from a slice which had been used in a recording experiment. Neurons fluorescently labelled with rhodamine-latex microspheres are visible within the graft.

Scale bar = 50µm

### Electrophysiological recordings

Slices taken from eight grafted rats and all four control rats yielded successful extracellular recordings. A total of 30 neurons were recorded from grafts whilst 38 neurons were recorded from normal control slices. None of the neurons from either group showed any spontaneous activity, but were all identified by their response to stimulation of the subcortical white matter. Figure 42 shows a typical response obtained for both grafted and control neurons after cortical stimulation. With each response there was always a delay of at least 2ms between application of a stimulus and the onset of the spike; only one spike was evoked per stimulus.

The density of the neurons in striatal grafts has been previously shown to be relatively normal (Isacson et al. 1985) but the number of grafted neurons identified electrophysiologically compared to those in control striatum was very much lower. This suggests that the cells were harder to find, which in turn implies that a smaller proportion of grafted neurons are responsive to cortical stimulation.

Table 5 shows the firing characteristics of both grafted and normal striatal neurons 'in vitro'. The current threshold required to produce a response was not significantly different between the two groups of neurons. For control neurons the mean threshold was  $0.51 \pm 0.22\text{mA}$  ( $n = 38$ ) compared to  $0.4 \pm 0.27\text{mA}$  ( $n = 30$ ) for grafted neurons. However, examination of the response latencies of the two groups of cells showed that only 26 of the 30 grafted neurons were directly comparable to those in the control group. The mean latency for the grafted neurons was  $4.78 \pm 2.45\text{ms}$  ( $n = 26$ ) compared to  $5.03 \pm 1.48\text{ms}$  ( $n = 38$ ) for the control neurons. The remaining four

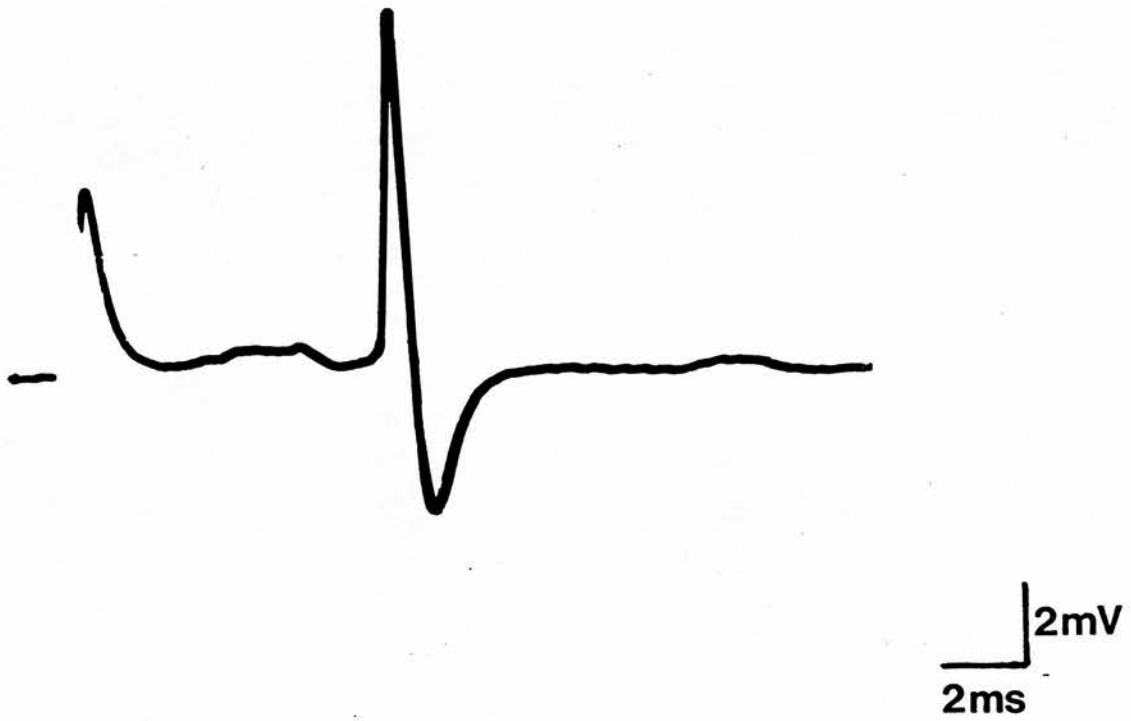


FIGURE 42. Typical response of a grafted striatal neuron to a single stimulus applied to the cortex.

TABLE 5. ELECTROPHYSIOLOGICAL CHARACTERISTICS OF GRAFTED AND NORMAL STRIATAL NEURONS.

	Control	Grafted
Threshold (mA)	$0.51 \pm 0.22(n=38)$	$0.41 \pm 0.27(n=30)$
Latency (ms)	$5.03 \pm 1.48(n=38)$	$4.78 \pm 2.45(n=26)$ $51.00 \pm 9.87(n=4)^*$
Rate of conduction( $\text{ms}^{-1}$ )	$0.13 \pm 0.03(n=38)$	$0.15 \pm 0.1(n=30)$
Inter-stimulus interval (ms)	$17.71 \pm 8.62(n=14)$	$5.6 \pm 3.21(n=5)^*$

\*  $p < 0.001$ .

graft neurons displayed distinctly longer latencies, the mean value being  $51.00 \pm 9.87\text{ms}$ . Figure 43 is a histogram showing the comparison between response latencies of grafted neurons and normal striatal neurons. This shows quite clearly the small population of neurons which responded with abnormally long latencies, with the result that graft neurons displayed a much wider range of responses than the normal control neurons. There was no apparent difference, however, between the current threshold required to elicit a response in these four and in the rest of the grafted neurons.

As mentioned in Chapter 3 normal striatal neurons show an initial excitation followed by a prolonged inhibitory phase after cortical stimulation (Herrling 1984; Marco et al., 1973; Morris et al., 1979). This inhibitory phase of the cortical response was tested by the method outlined in Chapter 3. The interval at which the second response was just still visible was the interval used to compile the data in the table and histogram. A typical response of grafted neurons to paired-pulse stimulation is shown in figure 44. The grafted neurons were found to have considerably shorter interstimulus interval thresholds than those in the control group (See figure 45) even though the population of neurons in this sample was fairly small. The mean interstimulus interval for the grafted neurons tested was  $5.6 \pm 3.21\text{ms}$  ( $n = 5$ ) as opposed to  $17.71 \pm 8.52\text{ms}$  ( $n = 14$ ) for control neurons (Mann-Whitney  $U = 5$ ;  $p < 0.001$ ). All 5 grafted neurons in this sample responded to cortical stimulation with latencies similar to those found for control neurons.

Rates of conduction for both groups of neurons were also calculated as outlined in Chapter 3. No significant difference

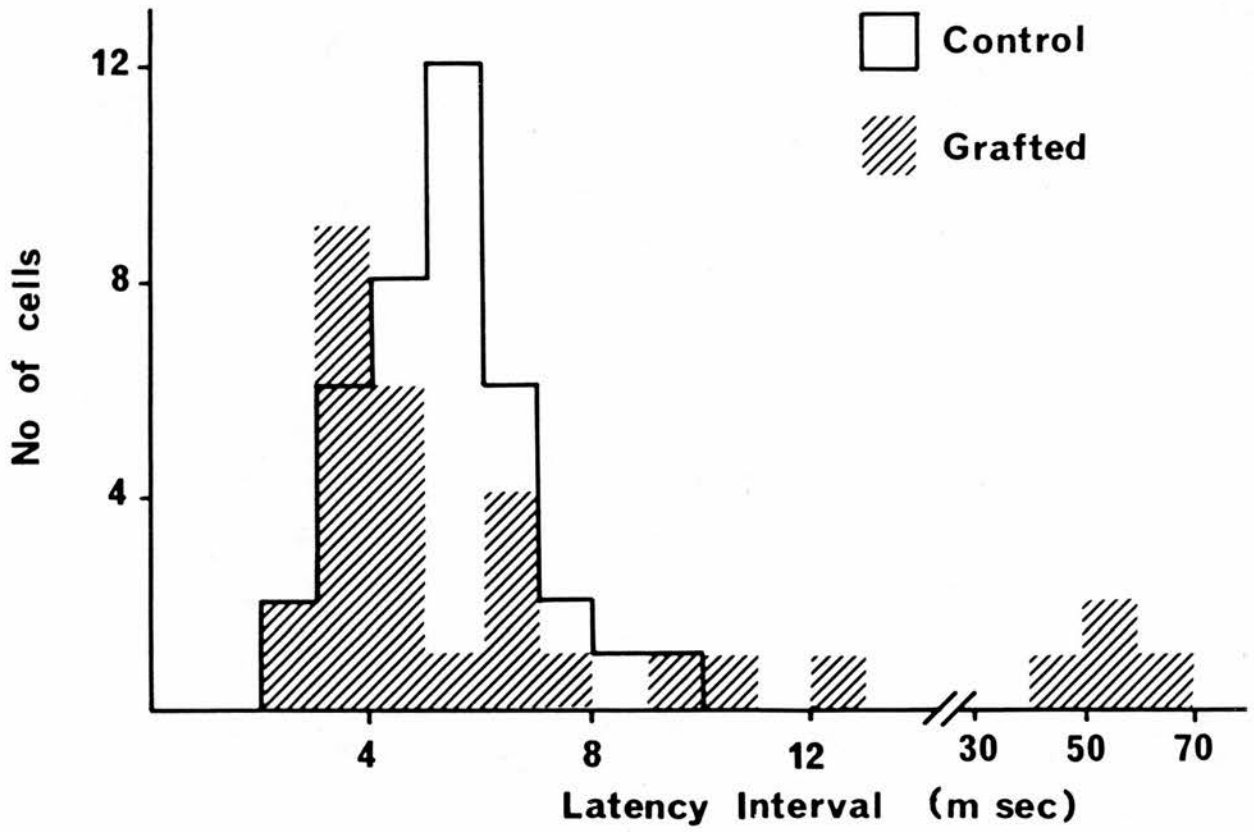


FIGURE 43. Latency histogram.

The response latencies of grafted and normal striatal neurons are compared.



FIGURE 44: Paired pulse stimulation of a grafted striatal neuron. The interval between stimuli applied to the cortex is 4ms. With shorter intervals the second response disappeared. (Compare with Figure 37).

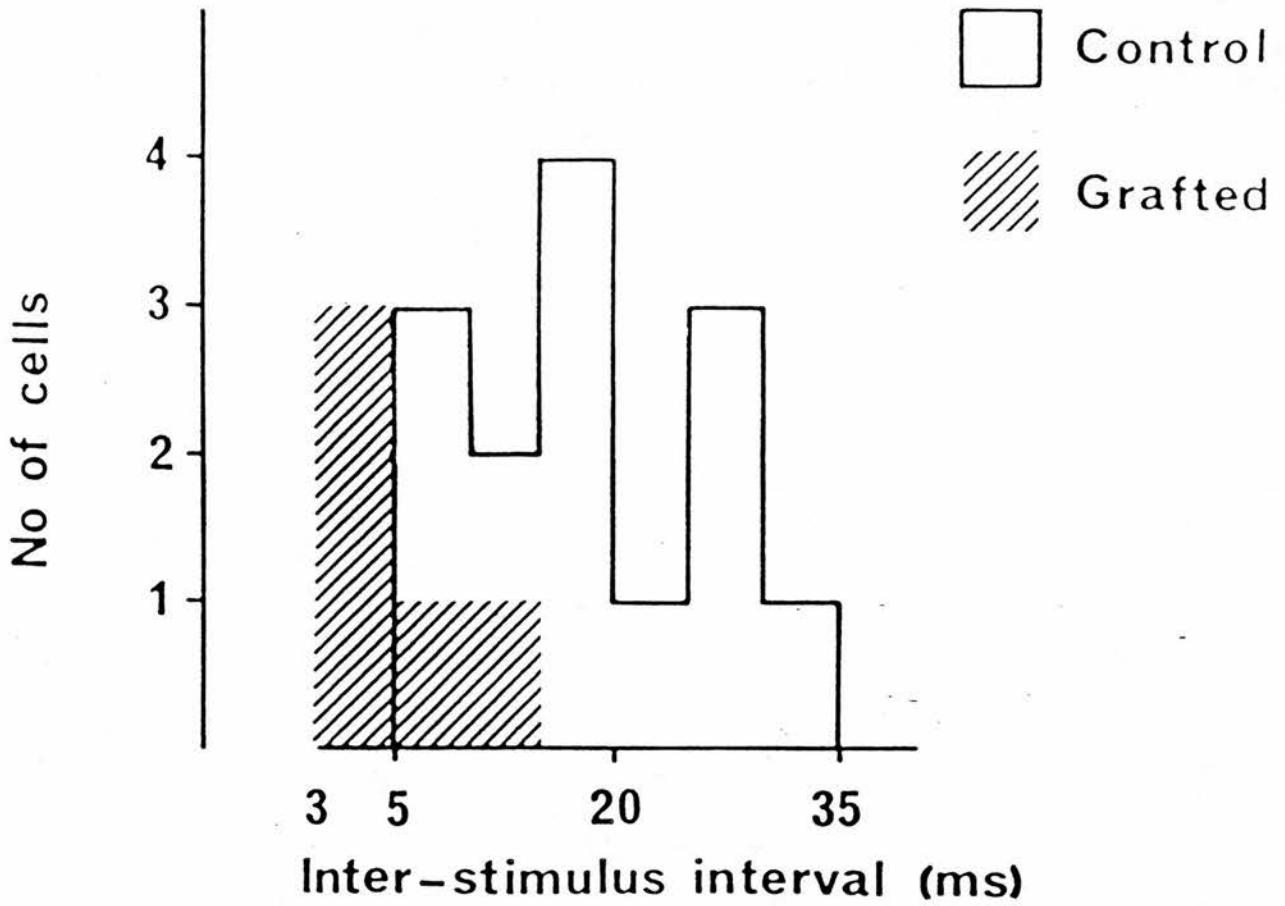


FIGURE 45. Interstimulus interval histogram. The shortest interstimulus intervals necessary to still elicit action potentials after cortical stimulation are shown on the graph. Shading indicates the responses of grafted neurons.



occurred between those of normal (mean =  $0.13 \pm 0.04\text{ms}^{-1}$ ,  $n = 38$ ) and grafted (mean =  $0.15 \pm 0.1\text{ms}^{-1}$ ,  $n = 30$ ) neurons and both were similar to the values obtained in Chapter 3.

#### Histological observations

The sections stained for TH were examined under a microscope (Olympus) and it was found that a considerable number of perikarya within grafts displayed TH immunoreactivity (Figure 46). In contrast to this the surrounding striatal tissue as well as the control tissue did not appear to contain TH-positive neurons. Those graft neurons displaying TH-immunoreactivity tended to be located mainly around the edges of the grafts, on the inner edge of the graft boundary. They tended to be very small (approximately  $10\text{--}15\mu\text{m}$ ) and round or slightly oval in shape with mainly one or sometimes two processes emanating from them. There was no obvious difference in the appearance of the TH-positive neurons between labelled and non-fluorescently labelled graft neurons, but the fact that the beads did not survive conventional mounting (alcohol dehydration, clearing in xylene and mounting) made detailed examination of the bead-labelled TH positive neurons difficult.

Numerous fibres displaying TH immunoreactivity were observed within the grafts, a number of which could be traced from the graft into the surrounding host tissue. These fibres however, did not travel very far into the host tissue and most stained fibres tended to meander around the margins of the graft.

In those sections with transplants containing fluorescently labelled neurons, few of these neurons appeared to contain TH-immunoreactivity. This was determined by observing sections

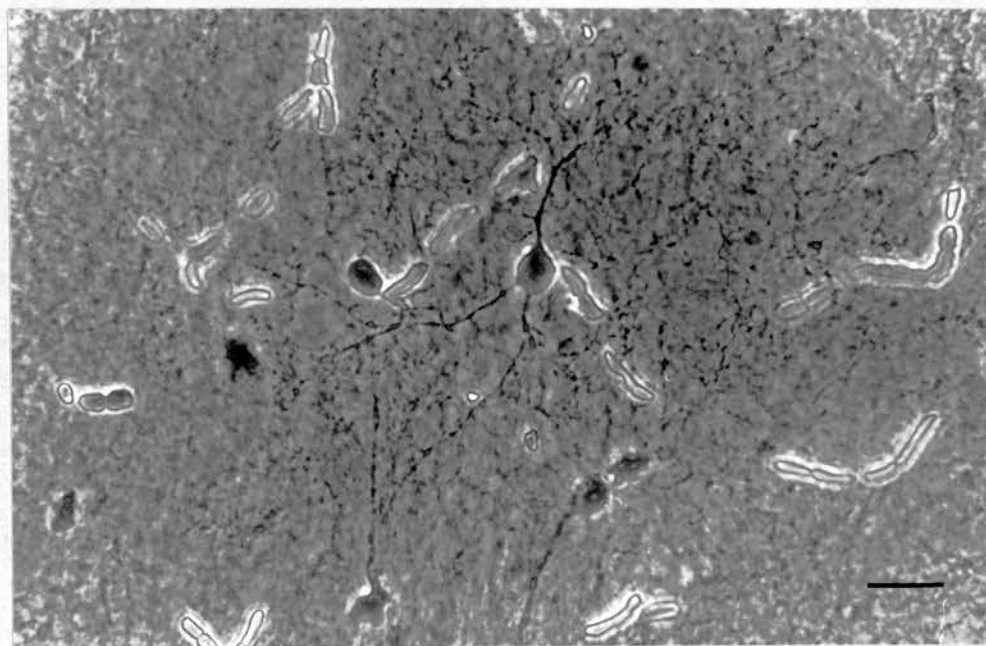
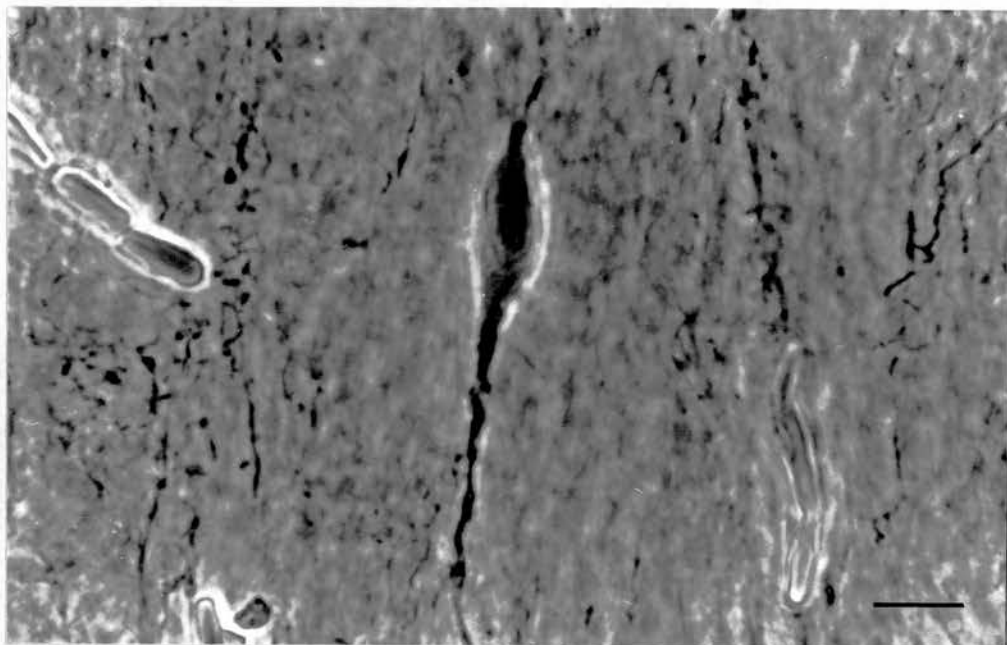


FIGURE 46. Examples of TH-immunoreactive striatal graft neurons. The 50 $\mu$ m sections have been stained using TH-immunohistochemistry.  
A. Bipolar striatal graft neuron. TH-positive fibres are also visible. Scale bar = 7 $\mu$ m.  
B. Several TH-positive neurons and fibres.

Scale bar = 20 $\mu$ m

Both photographs were taken under phase contrast.

which contained the fluorescent beads and which had also been stained for TH-immunoreactivity under a fluorescent microscope and switching rapidly to and from the fluorescence mode to normal viewing.

## DISCUSSION

The electrophysiological observations described in the preceding section provide clear evidence that a sub-population of neurons within striatal grafts receive a cortical input that is relatively normal in terms of the parameters of the primary response (see also Arbuthnott, MacLeod and Rutherford, 1985). This is in agreement with the findings of Wilson et al (1987) who recorded from grafted neurons while stimulating medial agranular cortex 'in vivo', although in the present study, some grafted neurons (13.3%) were observed to respond to cortical stimulation with significantly longer latencies than the rest of the grafted neurons and indeed those in the control group.

Those neurons with longer response latencies have been more noticeable because the sample sizes used were very small. If a larger population had been tested then perhaps the histogram obtained might have had a more normal distribution. Interestingly, the distribution of graft neuron response latencies observed was perhaps more similar to a histogram compiled for a similar experiment 'in vivo' (Brown, 1981) where the response latencies of cortically-driven striatal neurons recorded from normal rats displayed a similar range of latencies.

The abnormal responses to paired-pulse stimuli observed for the few grafted neurons tested suggest that they might in some way lack important intrinsic inhibitory regulation. The 'post-activation' inhibition of striatal neurons is a distinctive characteristic observed 'in vivo' (Herrling 1984; Marco et al., 1973) and has been thought to be a result of intrastriatal mechanisms. Park et al

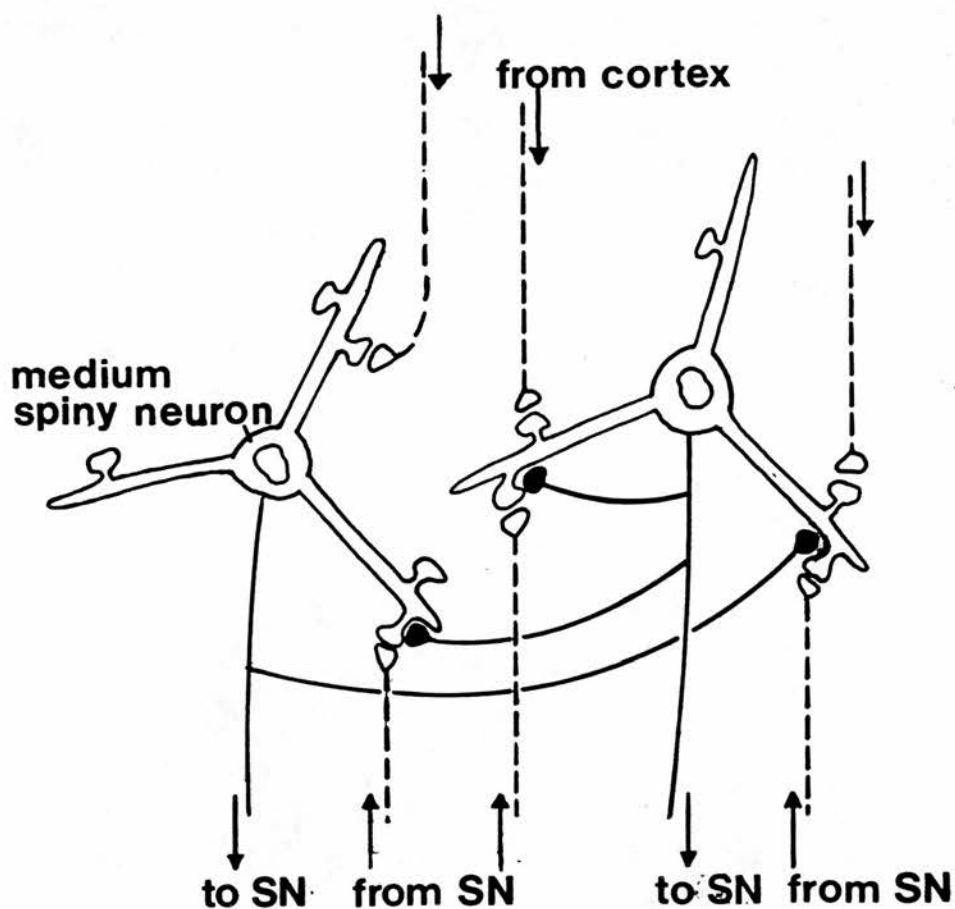
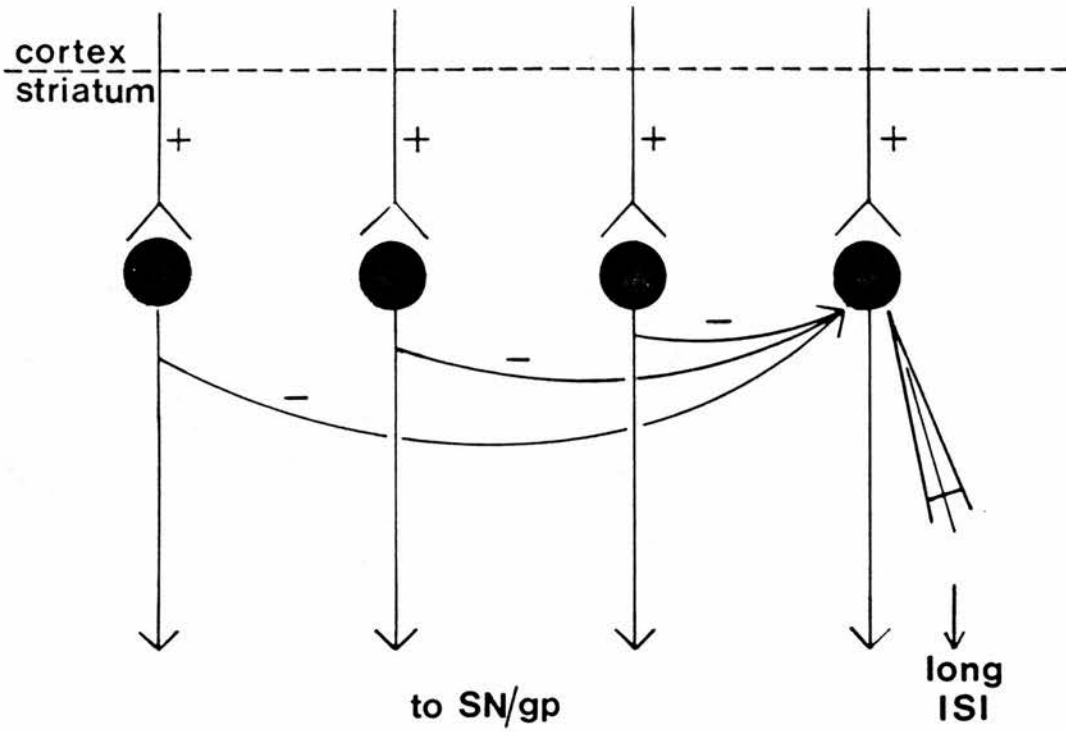


FIGURE 47. Diagrammatical representation of the proposed mechanism of recurrent inhibition in striatum. Only afferents from substantia nigra (SN) and cortex are shown. Redrawn from Park et al (1980).

(1980) have provided evidence that it may be a property of recurrent collateral activation providing a self-inhibition. Their experiments on cats demonstrated that an action potential triggered by intracellular current injection could decrease the amplitude of subsequent EPSPs evoked by substantia nigra stimulation (see Figure 47). Bicuculline was found to antagonise the effect which suggested that GABA may be the transmitter involved. The findings of the present study suggest that either this mechanism of recurrent inhibition does not develop properly within the grafts, or that the method of stimulation used (see Methods, p.164) does not activate the local inhibitory mechanism in the transplants. Obviously this warrants further investigation.

This paired-pulse phenomenon cannot simply be attributed to a lack of GABAergic neurons within the transplants since both postmortem (Isacson et al., 1984; 1985) and 'in vivo' biochemical studies (Sirinathsinghji et al, 1988) have recorded extensive GABA activity in the grafts and their efferent connections. An alternative suggestion arises from the reduced population of grafted striatal neurons which respond to cortical stimulation; out of 16 grafted animals only 30 neurons were recorded successfully compared to 38 from four control animals. As fewer neurons have the ability to respond to cortical stimulation in the graft (whether it be due to lack of cortical input or otherwise) collateral inhibitory influences might be very much reduced, as represented schematically in Figure 48. The observation that striatal graft neurons filled with HRP seem to have fewer collaterals present (C.J. Wilson, personal communication) lends support to this hypothesis.

### A. Normal animal



### B. Grafted animal

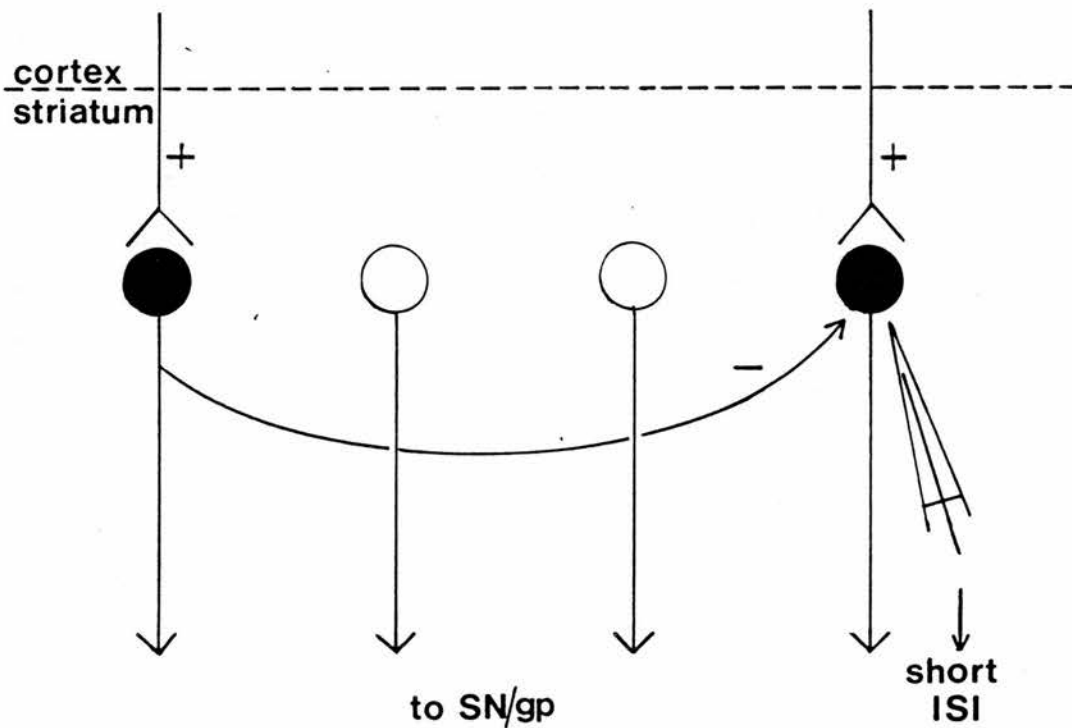


FIGURE 48. Proposed scheme to account for the lack of intrinsic inhibitory mechanisms within a striatal graft. Activated striatal neurons are represented by filled circles, non-activated by open circles.

#### Abbreviations:

+ excitatory input

- inhibitory input

ISI interstimulus interval

SN substantia nigra

gp globus pallidus

Another possible explanation comes from the suggestion that the development of striatal grafts becomes arrested at a relatively early postnatal stage in development (Isacson et al., 1987a; Dunnett and Bjorklund, 1987; McAllister et al., 1985; Zemanick et al., 1987; Walker and McAllister, 1987), showing immature neurochemical and morphological characteristics. The prominent IPSP which follows cortical stimulation as part of the EPSP-IPSP sequence has been found to develop relatively late in the postnatal period in kittens (Morris et al., 1979), suggesting that it may be immature in the grafts.

A possible problem which arises with this type of experiment 'in vitro' is that stimulation of striatal neurons may be direct rather than via the cortico-striatal pathway. This was discussed in detail in Chapter 3, but additional mention has to be made of the fact that at all times the stimulating and recording electrodes were positioned at least 1mm apart. As mentioned earlier, direct stimulation by current spread could only be detected in control experiments with stimulus intensities at least twice as high as any employed here. Furthermore direct stimulation usually resulted in response latencies which were less than 1ms, whereas all latencies observed here were more than 2ms.

Electrophysiological experiments carried out in other systems also lend support to the suggestion that viable graft-host or host-graft connections are formed. In a study by Arbuthnott, Dunnett and MacLeod (1985), electrophysiological recordings were made from DA grafts placed into the neocortex in direct apposition to the host striatum of rats which had previously received unilateral 6-OHDA lesions. Striatal, cortical and brain stem sites



within the host were stimulated and it was observed that neurons within the grafts responded to this stimulation. These findings supported the view that physiologically functional connections between host brain and graft can be established. In another such study (Wuerthele et al., 1981), DA-like neurons were identified in ventral mesencephalic grafts but the ventricular placements which were used greatly limited the detection of connections between graft and host.

Other electrophysiological experiments have been mainly concerned with the demonstration of graft-host connections. Stromberg et al (1985) demonstrated that nigral grafts placed within striatum could provide functionally significant reinnervation of striatum, an observation which was confirmed to some extent by the immunofluorescent demonstration of extensive networks of TH-positive fibres which extended 1-1.5mm from the grafts into the host striatum.

Only one other study of this nature has so far been carried out utilising the 'in vitro' brain slice technique (Segal et al., 1985). Septal grafts within the hippocampus were electrically stimulated and adjacent CA1 neurons were recorded. It was observed that over a period of several months, neurons located further away from the graft became activated by the stimulation suggesting that although slow, the growth of grafted septal neurons extends throughout the denervated hippocampus over a relatively prolonged period.

This observation might also help to explain the findings of a paper published by Walker and McAllister (1987). This study was carried out on striatal transplants in the striatum using WGA-HRP anatomical tracing techniques to detect connections between the

graft and host and vice versa. They found a lack of connectivity between graft and host brain at 1-2 months after transplantation but found labelling of fibres within the graft. Perhaps this was an indication of slow growth by the transplanted neurons, so that a similar study carried out by either (a) placing WGA-HRP in the frontal cortex and/or (b) repeating the experiment at different stages after transplantation might indicate that both afferent and efferent fibres had grown more towards their targets.

The electrophysiological observations outlined earlier (see Results) also provide support for some previous anatomical studies on the connectivity of striatal grafts (Pritzel et al., 1986). These investigators used WGA-HRP to study the afferent and efferent connection of the transplants and found that many of the normal afferent connections of the striatum (e.g. from substantia nigra and thalamus) had reformed connections with striatal grafts. However, a corticostriatal input was much harder to demonstrate; only weak staining was observed in all cases used in the study, including control tissue. As WGA-HRP is not the most sensitive anatomical tracer it might be possible to obtain clearer evidence for a cortical input to striatal grafts using the anterograde tracer PhAL (Gerfen and Sawchenko, 1984) and injecting it into the frontal cortex. (Recent experiments of just this type have confirmed the present results; Bjorklund, personal communication).

An important point to note is that the sample of neurons recorded from the striatal grafts were predominantly found within 200 $\mu$ m of the graft-host border. In contrast, most anatomical studies exclude cases where the tracer invades an area so close to the graft border in order to exclude problems associated with

leakage of the tracer out of the graft (Pritzel et al., 1986; Walker and McAllister, 1987). This therefore suggests that although striatal grafts receive a cortical input from the host brain, it may be very much localised to the border areas of the graft, and that this afferent system undergoes less extensive collateral regeneration than is seen, for example, in ascending monoaminergic systems (Pritzel et al., 1986).

Several anatomical studies have indicated that graft development becomes arrested at an immature stage (Isacson et al., 1987a; Zemanick et al., 1987; McAllister et al., 1985) such that the neurochemical staining pattern observed is that of an immature animal (Isacson et al., 1987a). Some groups have suggested that normal afferent projections are important to the developmental organisation of the striatum 'in situ' (Lanca et al., 1986; Walker and McAllister, 1987) such that if connections are not made, the graft remains 'frozen' in a state of immaturity. However, in the light of other anatomical and electrophysiological investigations, this seems unlikely and it is perhaps due to a reaction of the graft neurons to being resituated in a new and very different environment.

The observation that TH-positive neurons were present in the grafts (see results) is very puzzling. There could be several reasons for this:

- (a) the graft could contain tissue from other areas of the fetal brain besides the desired area.
- (b) the antibody used could be unspecific. This, however, seems unlikely as no other areas within the slices were seen to have labelled neurons.

Several other studies have made observations which suggest that

the findings here are valid. Specht et al (1978) demonstrated immunocytochemically the presence of TH in neurons within the ventricular zone of prenatal rat brain up until embryonic day 15 or 16 after which the TH-containing processes disappeared. They suggest that this transient presence may be significant in that these neurons may play some role related to proliferation or differentiation of neurons in the developing rat brain (see also Chun et al., 1987; Guillery et al., 1987).

Similarly Park et al (1986) (see also Kosaka et al., 1987) observed the expression of TH by E12-E15 neocortical neurons even after transplantation but these continued to be present for the life of the host animal. These neurons were found not to produce detectable levels of DA, NA or adrenaline so that the TH did not appear to be used for the production of catecholamine neurotransmitters. Therefore, it could be speculated that this enzyme remains present due to the graft neurons being placed in a developmentally abnormal state and may possibly play some role in directing the development of these neurons or indeed as a signal to in-growing connections. Alternatively, the antibody used in the present study could be detecting a related protein but not active TH.

Striatal grafts similar to those employed in the present experiments have been found to ameliorate lesion-induced deficits in complex cortically-dependent tasks such as delayed alternation learning, as well as in tasks that may simply reflect activation of striatal outputs (Isacson et al., 1986). This has been taken to suggest the possibility that striatal graft tissue may actually serve to reconstruct damaged cortico-striato-pallidal connections (Bjorklund et al., 1987). However, striatal grafts by no means

totally compensate for the loss of striatal function or indeed totally reconstruct damaged circuits. The mechanism or mechanisms by which they have their effects is still not clear although perhaps their ability to compensate for the various lesion-induced deficits has to be thought of in terms of a multiplicity of different mechanisms, not least of which is the re-establishment of host-graft connections, as has been suggested by the present results.

#### SUMMARY

1. Grafted neurons respond to cortical stimulation in a similar manner to normal striatal neurons.
2. Responses to paired-pulse stimulation suggest that grafted neurons lack local inhibitory regulation.

## CHAPTER 5

### Intracellular Recording of Striatal Neurons 'In Vitro': Effects of DA Administration

## INTRODUCTION

The dopaminergic nature of the nigrostriatal pathway originating from medium-sized substantia nigra zona compacta (SNc) neurons has been well established by various studies. For instance, fluorescent histochemical methods have demonstrated the presence of DA-containing neurons in the SNc and ventral tegmental area (VTA) with fine axons which project to all parts of the ipsilateral striatum (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971; Lindvall and Bjorklund, 1974). Furthermore, chemical or electrolytic lesions of the substantia nigra (SN) lead to degeneration of the pathway and loss of DA and dopaminergic terminals within the striatum (e.g. Hedreen and Chalmers, 1982; Poirier and Sourkes, 1965; Bedard et al., 1969; Hokfelt and Ungerstedt, 1969; Anden et al., 1964; 1965), identification of these neurons being made possible by the development of the Falck-Hillarp technique for fluorescent visualisation of catecholamines (Falck et al., 1962). Neurochemical studies (Portig and Vogt, 1969; Hedreen and Chalmers, 1972) have also shown that the release of DA from the ipsilateral striatum was increased during SN stimulation thus suggesting DA played some part in the influence of the SN on the striatum.

In spite of the wealth of evidence which points to the existence of a dopaminergic nigrostriatal pathway, the electrophysiological action of DA in the striatum has remained somewhat controversial. As a consequence of this, no definite agreement exists within the literature as to whether its actions are excitatory, inhibitory, or both. In parallel with the large number of studies carried out, a wide variety of experimental techniques and preparations have been

used on several species. This therefore might account to a certain extent for the diversity of the results obtained.

For the purposes of this review, studies of the action of DA on striatal neurons have been categorised as follows:

1. Examination of the spontaneous firing rates of striatal neurons after lesioning of the DA-containing cell bodies of the SN and their axons.
2. The responses of striatal neurons to electrical stimulation of the SN.
3. The effects of iontophoretic application of DA on the activity of striatal neurons.
4. Recent advances involving the study of the pharmacological aspects of DA-induced effects, both 'in vivo' and 'in vitro'.

All of these methods and their results are reviewed below. In addition, the effects of DA on cortically-evoked striatal activity will be discussed.

#### 1. Lesion studies

In an early experiment carried out by Ohye et al (1970), the spontaneous activity of the putamen and related structures was studied on both sides of the cat brain with chronic unilateral electrolytically-induced lesions of the median forebrain bundle (mfb). They found that the firing rates of neurons within the putamen on the lesioned side was increased compared to those on the contralateral side and in intact animals. It was concluded that this was as a result of the interruption of the nigrostriatal dopaminergic pathway and that DA was therefore exerting a predominantly inhibitory effect in the striatum. However, no



measurements of the striatal concentration of DA were made in this study.

Subsequent experiments were more rigorous in their approach to this problem. In one such study, Hull et al (1974) looked at the effects of different lesions in paralysed cats and behaving monkeys. Both groups were subjected to lesions of the mfb, SN or the area just dorsal to the SN and yielded similar results: an increase in the spontaneous firing rate of neurons in the striatum ipsilateral to the lesion. This alteration appeared to be independent of striatal monoamine concentrations as mfb and SN lesions led to reductions in striatal DA concentrations of 92% and 76% respectively, while lesions dorsal to SN had no effect.

These early studies concentrated on striatal neuron activity at 2-6 weeks post-lesion. In contrast, experiments carried out by Garcia-Rill et al (1980) examined the activity within the first few days after a mfb lesion. It was found that, unlike the studies described above, the spontaneous firing rate of neurons in the side ipsilateral to the lesion slowed significantly by 3 days post-lesion but returned to control values by day 7, while the firing rates of contralateral striatal neurons slowed progressively after the lesion. The observation that these changes were not correlated with changes in striatal DA concentrations was also confirmed, and led to the conclusion by these authors that there was little evidence in favour of DA acting as a straightforward neurotransmitter, whether excitatory or inhibitory.

Studies which have made use of electrolytic lesions have to be considered in terms of the specificity of the lesions produced. Whilst destroying the perikarya of the required pathway, fibres of

passage (including the axons of the neurons being studied) and indeed perikarya contributing to other, perhaps polysynaptic striatal afferent pathways, are also likely to be destroyed by this approach. There is also evidence to suggest the presence of a non-dopaminergic nigrostriatal pathway (e.g. Feltz and DeChamplain 1972; Hedreen, 1978; Fibiger et al., 1972; Ljungdahl et al, 1975; Van Der Kooy et al, 1987) so the contribution of damage to other pathways in the production of the overall effect may be a significant factor in the results obtained.

This problem was to a large extent overcome by the development and use of the neurotoxic agent 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968) which specifically destroys dopamine- and noradrenergic neurons. Injections of this toxin into the substantia nigra was found to cause anterograde degeneration of the whole nigrostriatal DA system whilst sparing fibres of passage and other non-dopaminergic neurons. Rats which received unilateral 6-OHDA lesions of the SN (Arbuthnott, 1974; Siggins et al, 1974; Ungerstedt et al, 1977; Schultz and Ungerstedt, 1978b; Schultz, 1982) were used to examine the firing rates of striatal neurons as a result of the lesion. Arbuthnott (1974) found that those striatal neurons ipsilateral to the lesion fired faster on average than those in the unlesioned side. While the firing rate within bursts remained unaltered, the interval between bursts was decreased. Siggins et al (1974) iontophoretically applied amino acids to locate normally silent neurons and discovered that the ratio of spontaneously active neurons to silent ones was increased in the lesioned side. This observation was confirmed by Schultz and Ungerstedt (1978b) and Schultz (1982) who also demonstrated that the neurons remained

TABLE 6 : THE EFFECTS OF SN STIMULATION ON THE ACTIVITY OF STRIATAL NEURONS

## (a) EXTRACELLULAR STUDIES

REFERENCE	ANIMAL	PREPARATION/ ANAESTHETIC	% NEURONS EXCITED	% NEURONS INHIBITED	% NEURONS UNAFFECTED
Frigyesi and Purpura 1967	cat	encephale isole	100	-	-
Albe-Fessard et al 1967	cat	encephale isole	32	68	-
McLennan and York 1967	cat	decerebrate	100	-	-
Connor 1968	cat	decerebrate	14	44	42
Feltz and MacKenzie 1969	cat	gallamine paralysis	100	-	-
Connor 1970	cat	decerebrate	16	47 \$	37
Feltz and Albe-Fessard 1972	cat	gallamine paralysis	?	61 \$	?
Gonzalez-Vegas 1974	rat	Urethane	22	46	32
Zarzecki et al 1976	cat	decerebrate	17	39	44
Zarzecki et al 1977	cat	decerebrate	10	38	52
Richardson et al 1977	cat	Urethane	100*	-	-
Katayama et al 1978	cat	gallamine paralysis	72	28	-
Norcross and Spehlmann 1978	cat	decerebrate	100	-	-
Fujimoto et al 1981	cat	$\alpha$ -chloralose	100	-	-
Hirata et al 1984	rat	Urethane	37	30	33

\* All neurons displayed an initial inhibition of their responses followed by excitation

\$ Some neurons responded in a biphasic manner (i.e. inhibition-excitation)

## (b) INTRACELLULAR STUDIES

REFERENCE	ANIMAL	PREPARATION	RESPONSES			% UNRESPONSIVE NEURONS
			% EPSP	%EPSP-IPSP	% IPSP	
Hull et al 1970	cat	gallamine paralysis	16	43.5	0.5	40
Kitai et al 1975	cat	gallamine paralysis	77	23	-	-
Kitai et al 1976	cat	Pentobarbitone	100	-	-	-
Kocsis and Kitai 1977	cat	$\alpha$ -chloralose	84	-	-	16
Morris et al 1979	cat	Halothane	-	100	-	-
Wilson et al 1982	rat	Urethane	-	100	-	-

supersensitive to applied DA for up to 12 months post lesion, although the changes in firing rate were temporary.

All these results were thus in agreement with the suggestion that DA may be an inhibitory transmitter in the striatum. However, the possibility remains that the loss of dopaminergic input to other areas, which in turn project to the striatum, might have been responsible for some of the effects observed. Bearing this in mind, it does not appear that lesion studies have made a conclusive contribution towards understanding the electrophysiological role of DA in the striatum although they have helped to establish that the nigrostriatal pathway does influence striatal neuron activity.

## 2. Studies involving substantia nigra stimulation

A wide variety of studies have been carried out in several different species and preparations to investigate the effects of nigral stimulation on striatal neuron firing. These are summarised in Table 6.

With this type of study a number of difficulties arise due to the uncertainties over the possible contribution of perinigral conductile pathways to the responses evoked in striatum by nigral stimulation. The morphology of the SN is such that the stimulation of this structure is also likely to involve excitation of the cerebral peduncle (which underlies almost three-quarters of its lateral extent), the medial lemniscus, (which is immediately superior to its lateral half), and of the red nucleus and perhaps other fibre tracts. Both the red nucleus and medial lemniscus, by virtue of their lower stimulation thresholds and higher conduction velocities, might make significant contributions to the "nigrally"

evoked effects. It is thus very unlikely that completely selective activation of the zona compacta neurons would be achieved. In addition, consideration of the possible existence of a non-dopaminergic pathway ascending from the SN has to be made. Furthermore it has been demonstrated that SN stimulation can elicit the antidromic activation of striatal output neurons (Kitai et al., 1975).

In spite of the problems involved in data interpretation, a large number of studies have demonstrated a striatal response to nigral stimulation. Depending on the type of recording used (i.e. intra- or extracellular) most studies showed that the initial response to SN stimulation was an EPSP, which was occasionally followed by an IPSP. However, these observations did not fit with the concept that DA acted as an inhibitory transmitter in the nigrostriatal pathway. Indeed, an early study carried out by Frigyesi and Purpura (1967) raised the question of the possible existence of functionally different nigrostriatal pathways subserving inhibitory and excitatory actions on striatal neurons.

A criterion for transmitter identification is the requirement that the putative transmitter should evoke the same subsynaptic responses as the natural transmitter (Wermann, 1966). However, very few of these studies actually compared the responses of striatal neurons to exogenously applied DA with those responses to SN stimulation. One such study which did compare these effects was carried out by Connor (1970) on decerebrate cats. It was found that the activity of 46% of striatal neurons tested was depressed by SN stimulation. These neurons were also consistently depressed by the iontophoretic application of DA, while both effects were antagonised

by  $\alpha$ -methyl DA. The results therefore tended to support the role of DA as an inhibitory transmitter of the nigrostriatal pathway.

McLennan and York (1967) and Gonzalez-Vegas (1974), on the other hand, found that iontophoretically applied DA depressed the effects of substantia nigra stimulation in the striatum.

Some confusion has arisen from these studies in terms of the short latencies of the nigraly evoked striatal responses, which are not entirely compatible with those expected for thin unmyelinated dopaminergic axons, especially when such extensive branching of these fibres occurs within the striatum (Anden et al., 1966; Collingridge et al., 1980). Conduction velocities obtained for peripheral c-fibres (Nishi et al., 1965) would imply that dopaminergic fibres would have a conduction velocity less than  $1\text{msec}^{-1}$ . Several studies in the rat have supported this prediction (Deniau et al., 1978; Guyenet and Aghajanian, 1978), which in turn would predict that response latencies of at least 19msec would be expected. EPSPs recorded after nigral stimulation have been found to have average latencies of approximately 4msec (Vandermaelen and Kitai, 1980; Preston et al., 1980), although in several instances, longer latency responses have been recorded extracellularly (Davies and Tongroach, 1978; Richardson et al., 1977). An estimate for response latencies in the cat, assuming a low conduction velocity similar to the rat, gave values of between 30 and 100msec (Moore and Bloom, 1978). This is in excess of the latencies reported for EPSPs in the cat (Kitai et al., 1976; Kocsis and Kitai, 1977). One recent study provides an explanation for these discrepancies. Wilson et al (1982) examined the intracellular responses of striatal neurons to SN stimulation in



TABLE 7 : STUDIES ON THE EFFECTS OF IONTOPHORETICALLY APPLIED DA ON STRIATAL NEURON FIRING

(a) EXTRACELLULAR STUDIES		PREPARATION/ ANAESTHETIC	TYPE OF NEURONAL ACTIVITY	RESPONSE OF RECORDED NEURONS TO IONTOPHORETIC APPLICATION OF DA (% OF TOTAL)			
REFERENCE	ANIMAL			+	-	0	BIPHASIC
Bloom et al 1965	cat	decerebrate/ various anaesthetics	spont spont	14 2.5	50 60	36 37.5	
McLennan and York	1967	cat	encephale isole	spont , DLH	9	60	31
York	1967	cat	encephale isole	spont , DLH	10	63	27
Herz and Zieglgansberger	1968	rabbit	gallamine paralysis	spont , DLH	-	70	-
Feltz	1970	cat	N20/methoxy- fluorane	spont , GLU	0	100	0
Connor	1970	cat	decerebrate	DLH , GLU	12.9	67	20.1
York	1970	cat	Penthrane/ decerebrate	DLH	44	31	-
Feltz and DeChamplain	1972	cat	DIAL , Urethane	GLU , ASP	-	43	-
York	1972	monkey	Penthrane	DLH , GLU	23	43	20
Gonzalez -Vegas	1974	rat	Urethane	DLH , GLU , spont	11	63	26
Siggins et al	1974	rat	Halothane	spont , DLH , GLU	5	90	5
Spehlmann and Stahl	1974	cat	encephale isole	spont	-	52	-
Spencer and Havlicek	1974	rat	DIAL/Penthrane	GLU	36	27	37
Woodruff et al	1974	rat	Urethane	DLH	0	100	0
Ben-Ari and Kelly	1975	cat	pentobarbitone/ Halothane	spont , GLU	4	92	-
Bevan et al	1975	rat	Halothane	spont	53	34	0
Spehlmann	1975	cat	decerebrate	spont/GLU	-	52/38	-
Stone and Bailey	1975	rat	Urethane	GLU , spont	6	72	22
Yarbrough	1975	rat	chloral hydrate	GLU	-	28	-
Stone	1976	rat	Urethane	GLU , spont	6	72	22
Woodruff et al	1976	rat	Urethane	DLH , spont	0	78	22
Zarzecki et al	1976	cat	decerebrate	GLU , spont	-	76	-
Ewart and Williams	1977	rat	DIAL/Urethane/ monethyl urea	spont	15.7	69	4.7
McCarthy et al	1977	rat	Urethane	spont , DLH	0	100	0
Biolac et al	1978a	cat	decerebrate	GLU , spont	7.7	67.3	25
Biolac et al	1978b	rat , cat	Urethane , decerebrate	GLU , spont	10.2	73.5	16.3

TABLE 7 CONTINUED.....

Davies and Tongroach	1978	rat	Urethane	DLH , GLU , spont	1.9	71	21.7	
Gmelin	1978	cat	N2O/O2 gallamine paralysis	spont	65	35	0	
Norcross and Spehlmann	1978a	cat	cerveau isole	striatal stimulation	83.3	14.3	2.4	
Norcross and Spehlmann	1978b	cat	cerveau isole	striatal stimulation	79.3	20.7	0	
Skirboll et al	1979	rat	gallamine paralysis	spont	0	100	0	
Arnauld et al	1981	rat	Urethane	spont , GLU	-	84	-	
Brown and Arbutnott	1983	rat	Halothane	spont , GLU	0 0	97 91	3 9	
Johnson et al	1983	rat	Urethane	spont , cortex stimulation	7	74	15	5
Chiodo and Berger	1986	rat	gallamine paralysis	spont GLU(low DA) GLU(high DA)	0 74 0	100 0 100	0 22 0	

(b) INTRACELLULAR STUDIES

Kitai et al	1976	cat	Pentobarbitone	-	31.4	0	-	
Bernardi et al	1978	rat	succinyl choline paralysis	SN stimulation GLU , spont	-	40	60	
Herrling and Hull	1980	cat	gallamine paralysis	cortex stimulation	0	100	0	
Mercuri et al	1985	rat	Pentothal	cortex stimulation	0	85	15	

ABBREVIATIONS :

- ASP : Aspartate-induced activity
- GLU : Glutamate-induced activity
- DLH : D,L-Homocysteic acid-induced activity
- spont : spontaneous activity
- +
- 
- 0



intact rats and after acute or chronic unilateral lesions of cerebral cortex or after combined cortex and unilateral thalamic transections, in effect removing all but the nigral input to the striatum. In intact animals, SN stimulation gave rise to a complex response with both excitatory and inhibitory phases. Chronic decortication eliminated the fast excitatory phase suggesting that this phenomenon was due to antidromic excitation of the cortical efferent axons with collateral branches synapsing on striatal neurons. After elimination of the other major inputs to striatum, 'pure' nigral stimulation gave rise to small EPSPs (with a mean latency of 10.4msec) which fitted much better with the predicted latencies for responses to nigral stimulation. No attempts were made, however, to determine the effects of directly administered DA on the striatal neurons in such preparations.

Thus, like lesion studies, experiments involving nigral stimulation have not led to the determination of a clear role for DA in the striatum.

### 3. Iontophoresis of DA

One way in which the effects of DA in the striatum can be studied with less involvement from outside influences such as polysynaptic inputs, is to iontophoretically apply the drug within the striatum. The large number of studies which have used this approach are summarised in Table 7. While the vast majority of these studies have pointed to a predominantly inhibitory effect of DA, several indicated that the main effect was excitatory (Kitai et al., 1976; Bevan et al., 1975; York, 1970; Gmelin, 1978; Norcross and Spehlmann, 1978a; 1978b).

Possible reasons for the discrepancies became clear on examination of the procedures involved in some of these studies. For instance, while they have been carried out on mainly rats and cats, a wide variety of anaesthetics and preparations have been used. In addition, some studies only examined the effects of DA on spontaneously active neurons which are not frequently observed within the striatum. This, therefore, would not appear to be a representative sample of striatal neurons. In many studies the use of excitatory amino acids to enhance spontaneous activity or to activate silent neurons also brings into doubt the validity of the observations as the effects of the amino acids might conceal the true effects of DA.

Discrepancies in the proportion of neurons excited by DA in various studies cannot easily be explained by the use of different anaesthetics, as different studies using the same anaesthetic produce very different results (e.g. Bevan et al (1975) and Siggins et al (1974); Gonzalez-Vegas (1974) and McCarthy et al (1977)). However, urethane-induced anaesthesia results in a very low spontaneous firing rate, which might affect the results obtained indirectly, as application of excitatory amino acids would be required to activate the vast majority of the striatal neurons. Several studies have shown that DA has an inhibitory effect on fewer of these neurons (Brown, 1981; Spehlmann, 1975).

The effects of DA on the orthodromic responses of striatal neurons are also mixed. Connor (1970) found that there was good correlation between the inhibition of striatal neurons by iontophoretically applied DA and that produced by SN stimulation. In addition, the antagonism of both DA and nigraly-evoked

depression of neuronal firing by  $\alpha$ -methyl DA was demonstrated, although no control excitation was used to show the specificity of the blockade. In contrast, a study carried out by Feltz and De Champlain (1972) showed that in normal animals the synaptically evoked nigrostriatal excitation was neither blocked nor facilitated by DA even if ejected with high currents or over prolonged periods. When these authors carried out the same experiments on cats with 6-OHDA lesions it was found that 8% of the neurons responding to nigral stimulation could be blocked by DA, perhaps indicating that they were hypersensitive to DA.

Facilitation of nigraly evoked responses has also been reported (Connor, 1970; Norcross and Spehlmann 1978; Zarzecki et al., 1976). Whilst finding the activity of some SN-stimulated neurons to be enhanced by DA, Zarzecki et al (1976) also observed that there was no correlation between the effects of iontophoretically applied DA and of the synaptically-mediated effects of the nigrostriatal pathway.

As mentioned earlier, several lines of evidence have suggested that DA is predominantly excitatory within the striatum. Kitai et al (1976) suggested that the inhibitory effects observed in other studies might be due to the application of DA on to nearby inhibitory neurons which in turn would suppress the activity of the recorded neuron. Although this does appear to be a viable explanation for extracellular studies, results from the large population of neurons recorded in many of the investigations outlined in Table 7 would have probably shown a higher proportion of excitations if this were the case. Consequently, this would also mean that the depressant action of DA would be prevented by

antagonists of inhibitory transmitters involved in the activity of these inhibitory neurons. In an attempt to determine whether this was the case, McCarthy et al (1977) iontophoretically applied the inhibitory transmitters glycine, GABA, taurine and DA on to striatal neurons and looked at the effects of strychnine and picrotoxin (glycine and GABA antagonists respectively) on the depression produced by the inhibitory neurotransmitters. It was demonstrated that they only inhibited the actions of glycine, and GABA and taurine, respectively and did not appear to be involved in the inhibitory effects of DA. Thus an involvement of inhibitory interneurons in the observed inhibitory effects of DA does not seem likely.

While various studies employing extracellular recording techniques have inferred that DA may have a hyperpolarising effect on striatal neurons (Feltz and De Champlain 1972; Herz and Zieglansberger, 1968) its true action on membrane potential and resistance requires the use of intracellular recording techniques. In comparison to extracellular studies the number of experiments employing intracellular recording and iontophoresis are few, not least because of the fact that great difficulty is involved with this type of recording.

Only four 'in vivo' studies coupled with DA iontophoresis have been reported to date. Those of Bernardi et al (1978), Herrling and Hull (1980) and Mercuri et al (1985) reported that DA gave rise to slow depolarisations coupled with a decrease in firing rate, although Mercuri et al (1985) also found that some neurons did not show any change in their resting potentials. On the other hand, Kitai et al (1976) demonstrated depolarisations similar to EPSPs

coupled with an increase in firing rate. Unfortunately the methodology of this study is open to question in that very short (5-100msec) pulses were used to apply DA and short interelectrode distances (20-40 $\mu$ m) were used. In addition, the depolarisations observed were very similar to EPSPs.

In an attempt to repeat the findings of Kitai et al (1976) Herrling and Hull (1980) iontophoretically applied DA approximately 50 $\mu$ m away from the recording electrode. Hyperpolarisations, which were accompanied by a decrease in firing rate, were observed in 30% of the recorded neurons. This was explained in terms of an effect of DA on the axon hillock resulting in a decrease in firing rate. On the other hand, the slow depolarisations which were also observed, were attributed to changes in the dendritic membrane. Neither Herrling and Hull (1980) nor Bernardi et al (1978) could demonstrate an increase in firing rate when DA was ejected in short pulses.

#### 4. Recent Approaches

A great deal of controversy and confusion has been generated from the types of experiments outlined in the preceding pages such that the electrophysiological role of DA in the striatum has remained largely unclear. The main problems which have arisen from these experimental approaches appear to be as follows:

- (i) Differences in the effects of DA on spontaneously active neurons and those activated by amino acids which could not be explained in terms of differing experimental approaches or anaesthetics used.

- (ii) Differences between the responses evoked by SN stimulation (i.e. endogenous DA) and iontophoretically applied DA. Some of the effects observed after iontophoretic application of DA and indeed SN stimulation did not appear to tie in with the concept of DA as an inhibitory transmitter in the striatum.
- (iii) The effect of iontophoretically applied DA could be either excitatory or inhibitory.

Several recent approaches have gone some way to resolve the various problems encountered in the earlier attempts to determine the role of DA. The problem of differential effects of DA on spontaneous and amino acid activated neurons was observed in several studies (Brown, 1981; Spehlmann, 1975; Johnson et al., 1983) such that its inhibitory effect may have been greater on one than the other. A recent study carried out by Chiodo and Berger (1986) may offer an explanation for this observation. They iontophoretically applied DA at two concentrations: at low levels, which usually produced little or no change in spontaneous firing rate, and at high levels which always significantly inhibited spontaneous discharge. With the lower concentration an enhancement of glutamate-induced excitation was observed, while the higher concentration gave rise to a significant decrease in this activity, thus suggesting that DA has a qualitatively different effect on striatal neurons depending on its concentration. Johnson et al (1983), using a similar approach, suggested that DA exerted a modulatory effect such that spontaneous activity was inhibited at "doses" which did not affect the activity evoked by cortical stimulation. It would thus appear that the intensity of iontophoretic current used would play an important part in determining the effects observed. The currents used by Chiodo

and Berger (1986) were much smaller than those used in earlier experiments, but when sufficiently large DA currents were applied approximately equivalent inhibition of spontaneous and amino acid-induced activity occurred. At this level therefore the action of DA appeared to be more consistent with that of an inhibitory transmitter. This might also explain the many observations of predominantly inhibitory action of DA on striatal neurons in previous experiments.

Another discovery which has helped to advance the understanding of DA actions in the striatum is that of different receptor types for DA. Recent receptor binding analysis has revealed that DA receptors may be classified into at least two subtypes. The first of these is the D1 receptor, which is linked to adenylate cyclase and secondly, the D2 receptor which is not linked and may actually inhibit the activity of the enzyme (Creese et al., 1983; Kebabian and Calne, 1979; Costall and Naylor, 1981; Seeman, 1980). This has resulted in the discovery and development of new, more specific agonists and antagonists for the DA receptor types, allowing for a better characterisation of the pharmacological effects of DA in the striatum. Based on these findings, Ohno et al (1985; 1986) carried out 'in vivo' electrophysiological studies using selective D1 or D2 receptor agonists and antagonists to resolve the discrepancy between the effects of iontophoretically applied DA and SN stimulation. 60% of SN-stimulated striatal neurons were inhibited by haloperidol and domperidone (D2 antagonists) but were not affected by the D1 antagonist SCH23390. It was also found that the increase in firing rate elicited by D2 agonists bromocriptine or LY171555 was only antagonised by the simultaneous application of D2 antagonists but



not affected during application of SCH23390. This therefore suggested that spike generation in striatal neurons upon SN stimulation is mediated by DA acting on postsynaptic D2 receptors.

Due to the problems encountered with iontophoresis of drugs and in the intracellular recording of striatal neurons 'in vivo', the 'in vitro' brain slice technique has been heralded as a means of overcoming such difficulties; the increased stability would improve and prolong intracellular impalements and the ability to bath apply drugs would at least allow their effects to be assessed quantitatively. For instance, Calabresi et al (1987) found that the effect of DA was inhibitory on all neurons tested 'in vitro', and was mediated by activation of D1 dopaminergic receptors. Biochemical studies have demonstrated that the D1 receptor in striatum shows low affinity for DA while the D2 receptor exhibits high affinity (Creese et al., 1983). Therefore, if D1 and D2 receptors are inhibitory and excitatory respectively then a low concentration of DA should have an excitatory effect while a high concentration would have the opposite effect. This clearly was not the case for the findings of Calabresi et al (1987) who obtained an inhibitory effect of DA which appeared to be mediated via D1 receptors.

A recent 'in vitro' study carried out by Akaike et al (1987) attempted to examine the possibility that high concentrations of DA would result in an inhibitory effect while low concentrations would produce an excitatory effect. They found that a low concentration of DA ( $1\mu\text{M}$ ) led to a depolarisation of the membrane and an increase in the spontaneous firing rate, together with an increase in the number of action potentials evoked by an intracellular depolarising



current pulse. These effects were antagonised by bath application of domperidone. On the other hand, higher DA concentrations (100–500 $\mu$ M) inhibited spontaneous and current induced neuronal firing (without apparent effects on resting membrane potential) by raising the current threshold for spike activation. This inhibitory effect was antagonised by the D1 antagonist SCH23390.

The observation that application of a low concentration of DA resulted in a depolarisation which appeared to be mediated via D2 receptors led the authors to suggest that the D2 receptors were located postsynaptically on dendritic spines, while the effects of high concentrations of DA were suggested to be mediated by D1 receptors extrajunctionally located on the soma. These observations thus gave support to the findings of Herrling and Hull (1980) outlined earlier. However, no attempts were made to mimic the effects of DA with receptor specific agonists, and the use of domperidone was somewhat of a surprise choice considering the availability of more specific agents.

The study carried out by Akaike et al (1987) might explain the differences between the effects of SN stimulation and iontophoretically applied DA in the striatum, as the amount of DA ejected by iontophoresis would usually tend to be much greater than that released by SN stimulation so that the predominant effect would be inhibitory as opposed to the excitation usually observed with SN stimulation. However, a recent neurochemical study (Kelly and Nahorski, 1987) showed that released DA can activate both D1 and D2 receptors, seen as stimulation and inhibition of cyclic AMP accumulation respectively.

Thus the effects of DA still remain somewhat unresolved although it now appears that it does not have just a simple inhibitory or excitatory role in the striatum. The possibility of interaction between different DA receptor types in the mediation of DA effects is a factor which cannot be excluded.

#### Effects of DA on cortically-evoked striatal activity

Various anatomical and neurochemical studies have demonstrated that nigrostriatal and prefrontostriatal paths converge within the striatum (Beckstead, 1979; Bouyer et al., 1984; Kemp and Powell, 1971; Kornhuber and Kornhuber, 1983; Godukhin et al., 1984). While Kitai's group (Kocsis, Sugimori and Kitai, 1976) had demonstrated a convergence of cortex and nigral inputs on to single striatal neurons, electrophysiological evidence for an interaction between DA and the cortical input to striatum was not proposed until a few years later (Olpe and Koella, 1978). They observed that repetitive unilateral stimulation of the SN inhibited striatal potentials evoked from ipsilateral rostral cortex, and intraperitoneally administered neuroleptics (e.g. haloperidol, chlorpromazine) antagonised these inhibitory effects. However, their experimental approach could not resolve the exact mechanism which brought about the inhibition of cortically evoked potentials.

Several other studies have demonstrated the effects of DA on activity evoked by cortical stimulation (Hirata et al., 1984; Brown and Arbuthnott, 1983; Johnson et al., 1983; Mercuri et al., 1986; Fujimoto et al., 1981). The excitatory response of striatal neurons after cortical stimulation was shown by Hirata et al (1984) to be more affected by the iontophoretic application of DA than was

spontaneous activity. Similar results were obtained following nigral stimulation prior to cortical stimulation. This indicated a possible presynaptic action of nigrostriatal terminals on corticostriatal fibres (Hirata et al., 1984). In contrast, however, Johnson et al (1983) found that the spontaneous activity of striatal neurons was inhibited at doses of DA which did not affect the activity evoked by cortical stimulation. This study employed pressure ejection as the method of DA application and so this may account for the different results (but see Brown and Arbuthnott, 1983).

The discovery of the existence of a population of DA receptors which were located on the terminals of cortical afferents and which appeared to be of the D2 type (Schwartz et al., 1978) prompted Brown and Arbuthnott (1983) to investigate the interaction of DA with corticostriatal transmission on a pharmacological basis. DA applied iontophoretically to the striatum of the rat was found to inhibit spontaneous, glutamate-induced, and cortex-evoked activity. However, a DA current sufficient to markedly depress spontaneous activity left cortical excitation unaffected. Sulpiride (a D2 antagonist) failed to antagonise the depressant action of DA on spontaneous or glutamate-induced activity, but was found to facilitate the responses to cortical stimulation. Simultaneous application of DA with sulpiride blocked this effect. These results strongly suggested a presynaptic site of action on corticostriatal terminals. A similar experiment carried out by Vives and Mogensen (1986) confirmed that the effects of nigral conditioning stimuli on the excitatory responses to cortical stimulation were mediated by D2 receptors on terminals.

A recent study by Mercuri et al (1985) examined the effects of DA on cortically-evoked EPSP-IPSP sequences. Intracellular recordings indicated that DA decreases the amplitude of EPSPs and IPSPs by reducing the membrane conductance measured at their peaks, while the input resistance of the non-synaptic membrane was not modified. DA also inhibited spike frequency resulting from depolarising pulses and decreased the depolarisation-induced release of glutamate at the presynaptic site. The membrane potential of some neurons was slowly depolarised by DA whilst in other neurons it remained unchanged (see Herrling and Hull, 1980). The results suggested that DA had both a presynaptic and post-synaptic effect; it decreases the input from cortex via a D2 mechanism and also the membrane responses to this input by decreasing intrinsic membrane excitability. These authors also favoured a complex neuromodulatory role for DA in the control of striatal neuron firing.

In spite of these findings the pharmacological basis of the dopaminergic effects on cortical stimulation have been limited in the 'in vivo' preparation by the difficulty in applying substances quantitatively within the brain and also in the lack of intracellular recordings which would have defined the site of action more precisely.

In the present study the 'in vitro' brain slice preparation was used in conjunction with intracellular recording techniques in an attempt to overcome these limitations. When this series of experiments was initially started few studies had been carried out in the striatum 'in vitro', and fewer still on the effects of DA therein. As described in Chapter 3, a successful slice preparation was developed which maintains most of the corticostriatal afferents

intact. Therefore at the outset, the main goals of the study were to characterise the properties of striatal neurons intracellularly and to examine the pharmacological effects of DA on these properties.

## METHODS

### Animals

Male Albino rats weighing 120–150g were used throughout the experiments.

### Preparation and maintenance of slices

These procedures were carried out as described in Chapter 3.

### Microelectrodes

Recording microelectrodes were manufactured from 1.5mm diameter capillary glass tubing (as outlined in Chapter 3) but for these experiments electrodes of 80–120 MOhms were used.

### Filling solutions

Several solutions were tried, all of which were fairly concentrated (1–3 Molar) in order to obtain a high concentration of ions at the tip and hence a low electrical resistance (Purves, 1981).

Using a fine bore needle the electrodes were filled directly from the stem. The filling solutions used were usually filtered before they were injected into the electrodes by a fine Millipore disposable filter attached to the filling syringe. This went some way to prevent impurities from blocking the electrode tips.

The most successful filling solutions tried were 3M potassium chloride (KCl) and 2M potassium acetate (KAc). However, the disadvantage of using such concentrated solutions is that they lead to a large diffusional leak out of the electrode tip, the problem being more acute with KCl than KAc due to the upsetting effects of

$\text{Cl}^-$  ions on the  $\text{Cl}^-$  equilibrium potential, such that the sign of inhibitory postsynaptic potentials can be reversed (see Thomas, 1978). It was therefore decided that 2M KAc would be used for all experiments reported here.

Tip potentials were checked before each penetration commenced (see Purves 1981). If these were too large then the electrodes were discarded.

### Electrical Recording

Throughout the study several different amplifiers were used. In every experiment the electrode resistance was carefully counterbalanced by means of an active 'bridge' circuit in the amplifier. In the later experiments, from which most of the records were made, an Axoclamp 2A was used so that the electrode capacitance could also be compensated for. The 'sample and hold' output of the amplifier was monitored while the amplifier was set in 'current clamp' mode. The capacitance neutralisation could then be adjusted to obtain the best square wave response from the 'sample and hold' circuit and hence the best noise level in the current and voltage switched output from the amplifier. Current and voltage were displayed on a dual beam Tektronix storage oscilloscope. Stimulation and timing were controlled by a Digitimer 4005 timing unit and several isolation units provided timed, constant voltage pulses. One of them supplied the hyperpolarizing pulses which were used to search for neurons by detecting changes in input resistance. Another timing pulse allowed 1msec of "decompensation" of electrode capacitance neutralisation, and thus could "ring" the electrode into cells. An isolation unit connected through a

constant current device allowed stimulation of the slices at times associated with oscilloscope triggering and intracellular current stimulation.

In some experiments the signal was tape recorded (Tandberg four-channel FM tape recorder) for subsequent analysis.

#### Drug solutions for bath application

Dopamine ( $1\mu\text{M}$ ) was made up in control medium to which  $1\mu\text{g/ml}$  of ascorbic acid had been added in order to prevent oxidation of the DA. Oxidation could be readily detected by the change in colour of the DA solution from clear to pink. For this reason, these solutions were always made as required and discarded at the end of each experiment. The addition of ascorbic acid alone to the bathing medium had no effects on the resting membrane potential, the shape and height of the action potentials or the firing frequency in any of 6 striatal neurons tested in 5 slice preparations.

Tetrodotoxin ( $1\mu\text{M}$ ) and TEA ( $5\text{--}20\mu\text{M}$ ) were made up in distilled water and were added to the inflowing control medium as required.

All drugs were supplied by Sigma.

To examine the effects of changes in extracellular  $\text{K}^+$  concentration on the AHP the concentration of potassium within the bathing medium was altered such that when a total concentration of  $1.5\text{mM}$   $\text{K}^+$  was required, KCl was replaced by an equivalent amount of NaCl in order to keep the medium in osmotic balance. Conversely, to achieve a total concentration of  $9.5\text{mM}$ , extra  $\text{K}^+$  had to be added. However, addition of KCl or  $\text{KH}_2\text{PO}_4$  would have altered the osmolality of the solution so  $\text{K}^+$  was added as the salt of L-Gluconic acid, an impermeant anion, to maintain the balance.



### Preliminary Technical Considerations

We obtained stable, successful intracellular recordings from striatal neurons. Well-impaled neurons remained consistent in resting membrane potential and response properties over prolonged recording periods. Resting potentials recorded were at least  $-65\text{mV}$  and spike amplitudes were always greater than  $70\text{mV}$  whether triggered from intracellular or cortical stimulation. Impaled neurons which did not conform to these criteria were discarded, although this happened rarely.

It was hoped that this technique would help to resolve the pharmacological actions of DA in the striatum, and on the responses to corticostriatal stimulation in particular. However, when DA was applied via the bath, the neurons very rarely recovered from the changes which followed. Several factors seem likely to be responsible for this.

1. The 'dead space' in the bath used in these experiments was rather large (approximately  $3\text{ml}$ ). This implied that the onset of action of an applied drug and recovery from its effects might take a considerable length of time (i.e. minutes as opposed to seconds) due to the length of time the drug would take to enter the recording chamber and reach the slice. This in turn is controlled to a certain extent by the flow rate. However, different flow rates did not have any effect on the recovery of impaled neurons.
2. The volume in the recording chamber (i.e. without the contribution of inlets and outlets) was in itself quite large and so it seemed possible that drugs might not mix fully in this area. This was tested by dropping a small volume of dye (equivalent to the volume of DA solution usually applied) into the constant head

device. It was observed that the dye took approximately 20-30 seconds to reach the recording chamber, a value which was almost ten times faster than the onset of effects observed after bath application of DA. Removal of the dye took considerably longer; one hour after initial application, dye was still visible within the recording chamber. It seemed that while the movement of drug in the inlet and outlet tubes was fairly rapid, mixing within the recording chamber was very slow.

3. The iontophoretic application of glutamate led to a rapid onset excitatory effect from which the recorded neuron recovered very rapidly. This therefore suggested that DA application by a similar route might be more successful.

#### Drug solutions for iontophoresis

L-glutamate (0.2M, pH7) and dopamine (0.5M, pH 4) were dissolved in distilled water and the pH adjustment made with hydrochloric acid and sodium hydroxide. In addition, 0.2mg of ascorbic acid was added to the DA solution to prevent oxidation.

Several studies have demonstrated that the effects of iontophoretically applied ascorbic acid are excitatory (Gardiner et al., 1985; Ewing et al., 1983), an effect which was never observed when DA (plus ascorbic acid) was being ejected. Similarly, when an ejection current of the same magnitude as that used to iontophorese DA was applied to the NaCl balancing barrel, no effect was observed.

## RESULTS

Various properties of striatal neurons were characterised intracellularly and the effects of DA on some of these properties were examined. Preliminary studies were also carried out on the ionic mechanisms underlying the activity of these neurons.

### (i) Membrane properties

Intracellular records were obtained from a total of 96 striatal neurons, which were maintained in stable conditions for at least 30 minutes prior to any measurements being made. These neurons had resting membrane potentials of at least  $-68\text{mV}$  (mean  $\pm$  SD,  $-82.18 \pm 6.96\text{mV}$ ). The measurement of resting potential was made upon penetration and again after withdrawal of the electrode from each neuron after all intracellular data was obtained. No spontaneous activity was observed; rather, action potentials were elicited by rectangular depolarising pulses of current applied intracellularly. The mean threshold for spike activation was  $0.74 \pm 0.41\text{nA}$  ( $n = 96$ ). Action potential duration was less than  $1.5\text{ms}$  in all cases and all were at least  $70\text{mV}$  in amplitude (mean  $\pm$  SD,  $80.81 \pm 7.63\text{mV}$ ,  $n = 59$ ). In most neurons, action potentials appeared to be triggered from small, slowly rising potentials (Figure 49B) and were succeeded by small after hyperpolarisations of approximately  $8\text{--}10\text{mV}$  in amplitude (Figure 49C).

Table 8 shows that DA application did not affect resting membrane potential or spike amplitude in any of the 26 neurons tested. However, it did appear to have an inhibitory effect on all the neurons which was observed as a decrease of the number of spikes

TABLE 8. Intracellular recording of striatal neurons: A comparison of various parameters before and after the application of DA.

PARAMETER	CONTROL	AFTER DA
Resting membrane potential(mV)	$-82.18 \pm 6.96$ (n = 96)	$84.5 \pm 7.39$ (n = 26)
Spike threshold (nA)	$0.74 \pm 0.41$ (n = 96)	$1.27 \pm 0.57$ (n = 26)***
Spike amplitude(mV)	$80.81 \pm 7.63$ (n = 59)	$82.23 \pm 7.05$ (n = 13)
Input resistance(MOhms)	$21.79 \pm 4.70$ (n = 28)	$19.51 \pm 4.75$ (n=16)*
Number of spikes per stimulus x 2 threshold	$30.3 \pm 11$ (n = 16)	$38 \pm 11$ (n = 16)**
x 3 threshold	$39.96 \pm 12.57$ (n = 9)	$39.14 \pm 12.41$ (n = 7)*

\* p > 0.05

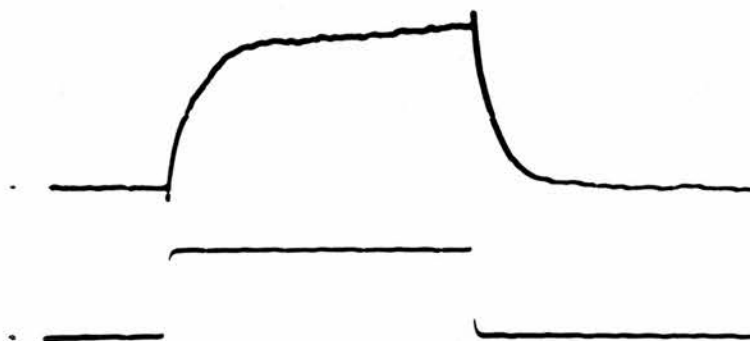
\*\* p < 0.01

\*\*\* p < 0.1

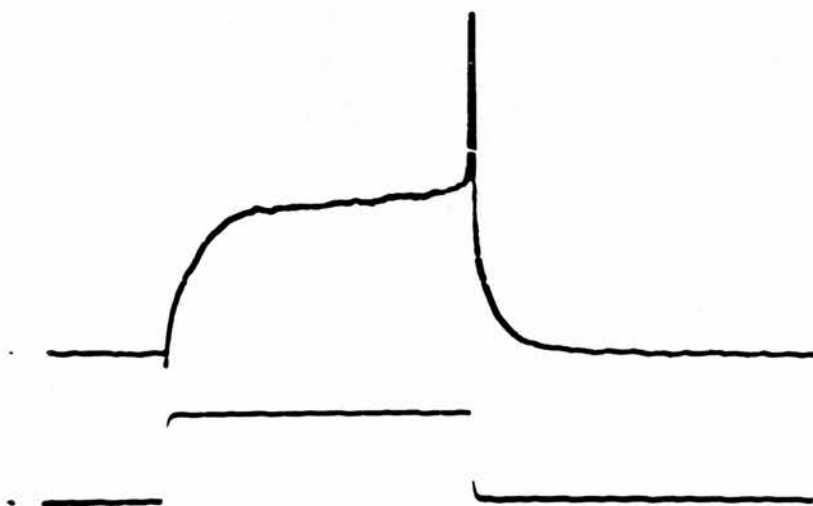
FIGURE 49. Intracellular current injection.  
A. Subthreshold for spike activation.  
B. Spike threshold  
C,D. Increase current intensity results in an increase  
in the number of spikes evoked.  
Responses of striatal neurons were recorded in  
current clamp mode so that the spikes have been  
filtered.

A

-221-



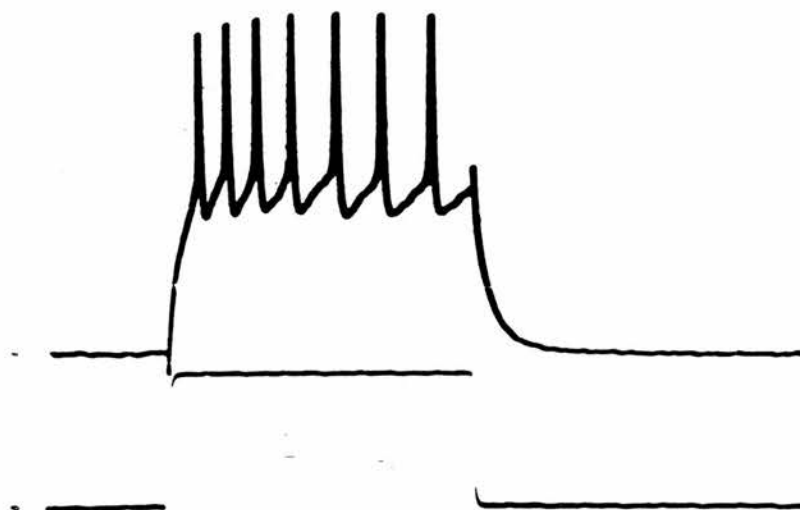
B



C

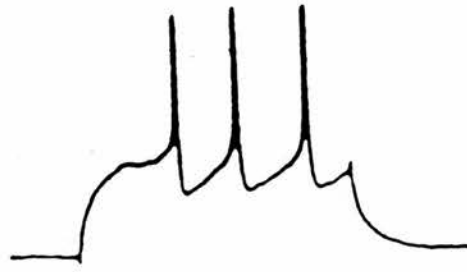


D



1nA  
20mV  
20ms

Control



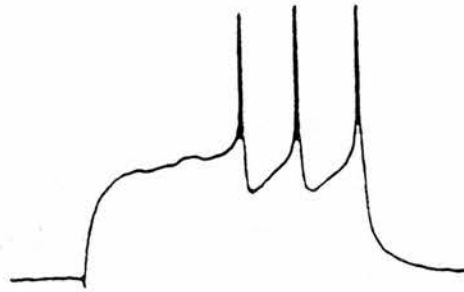
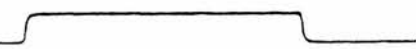
0.4nA



Dopamine (DA)  
(25nA, 3mins)



0.4nA



0.5nA



20mV  
1nA  
20ms

FIGURE 50. Effects of iontophoresed DA on action potentials evoked by an 80ms wide depolarising current pulse applied to a striatal neuron.

The same number of spikes as observed in the control situation can still be evoked after DA by increasing the stimulus intensity. No changes in spike height or membrane potential were observed after DA application.

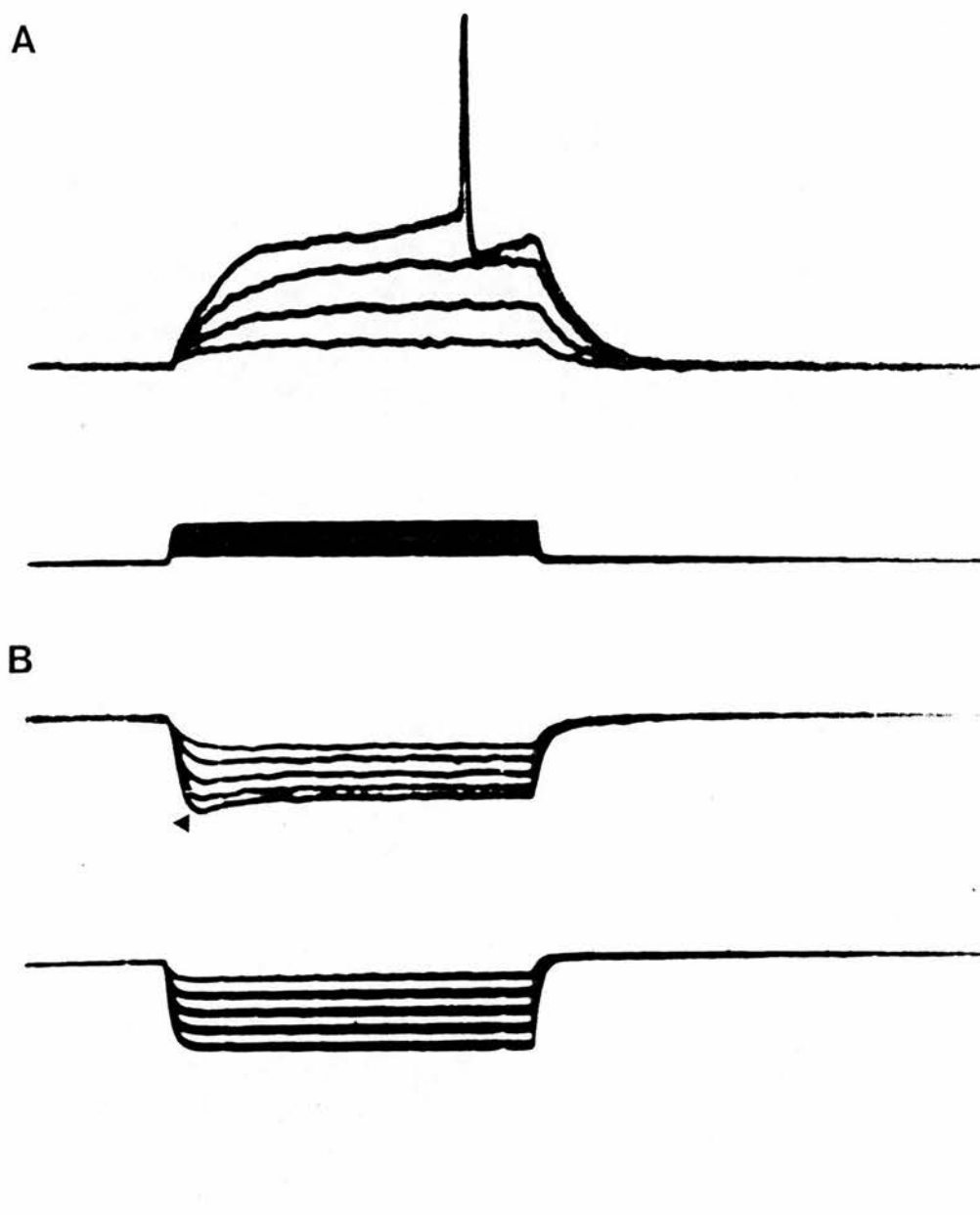


FIGURE 51. Responses of a single striatal neuron to injections of varying current intensities.

A. Depolarising currents up to threshold level

B. Hyperpolarising currents. The prominent voltage "sag" is shown by the arrow.

Scale: 20ms, 20mV and 1nA



evoked by depolarising pulses, with the spikes themselves appearing unmodified. An increase in the amount of current injected restored spike firing indicating that DA acted on spike threshold (Figure 50), but no spontaneous recovery of activity was observed in any of the neurons tested. The mean value for spike threshold after DA application was  $1.27 \pm 0.57\text{mA}$  ( $n = 26$ ), which was significantly higher than the control value ( $p < 0.1$ , paired t-test). These measurements were made at approximately 5 minutes after the onset of any observed effect of DA in order to standardise the measurement of activity changes.

#### (ii) Current-voltage relationships

The input resistance of individual striatal neurons was determined from the potential shifts across the membrane during the passage of inward and outward rectangular current pulses (80ms wide) of known intensity, and which were subthreshold for spike activation in the depolarising direction. Measurements were based on several current intensities passed in both directions. In each case the reliability of an estimate was confirmed by repeated measurements. Extreme care was taken before and during these measurements to adjust the bridge balance of the preamplifier.

Fig. 51 shows the responses of one particular neuron to several different currents. These were applied in increments of  $0.1\text{nA}$  in the depolarising direction to values just below the firing threshold, and in the hyperpolarising direction to  $-2.0\text{nA}$ . The slope of such a current-voltage relation was usually linear over the middle portion of the plot (i.e. around zero current), but for all neurons the application of higher current intensities in both directions led to

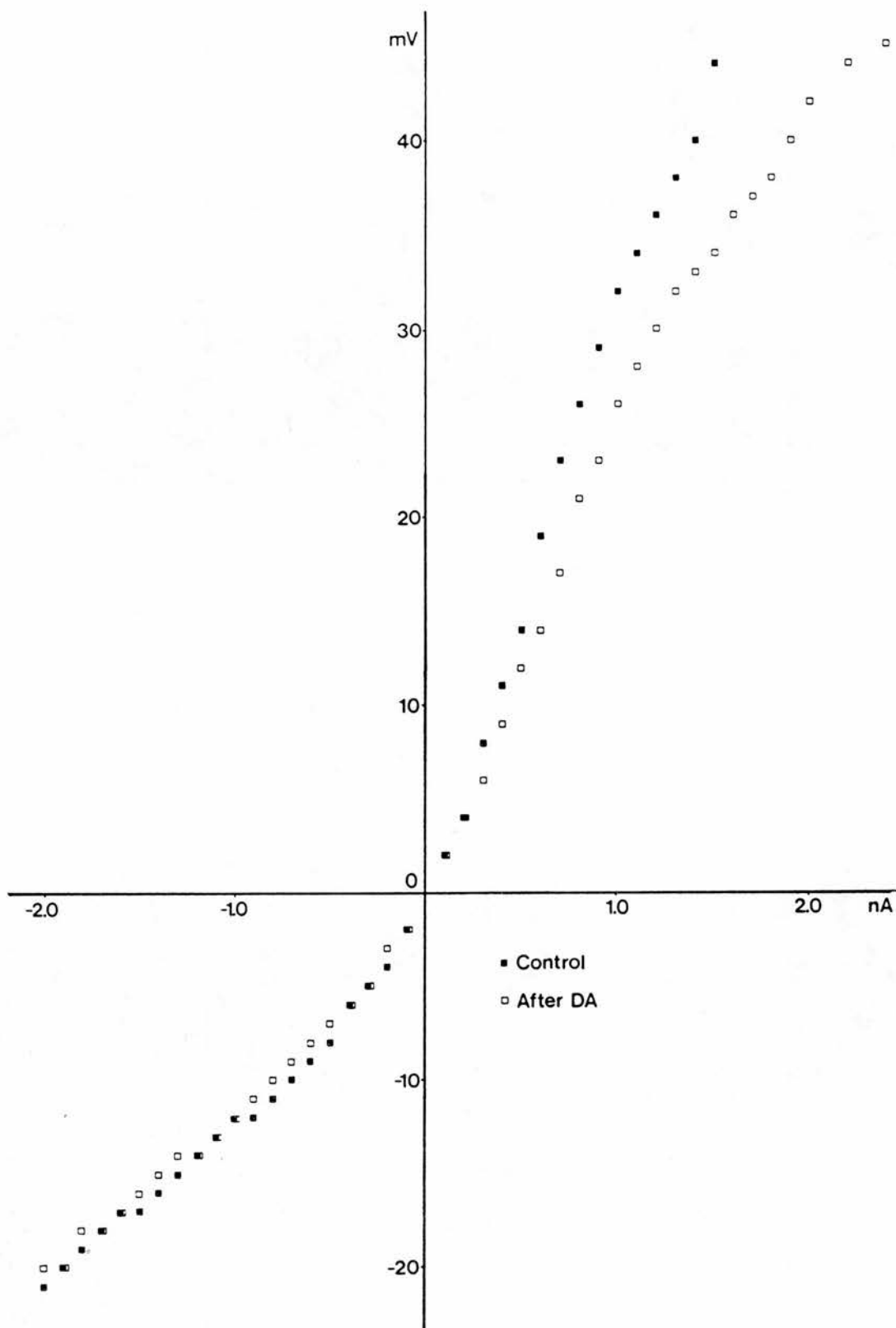


FIGURE 52. Current-voltage relationship of a striatal neuron before and after DA application. The voltage responses of a single striatal neuron to injections of depolarising and hyperpolarising currents were measured. In the hyperpolarising direction, steady state voltages have been used to produce the graph. "Sag" voltages have been omitted. The input resistance of this neuron was  $20\text{M}\Omega$ . The effect of DA on the current-voltage relationship is indicated by the open squares.

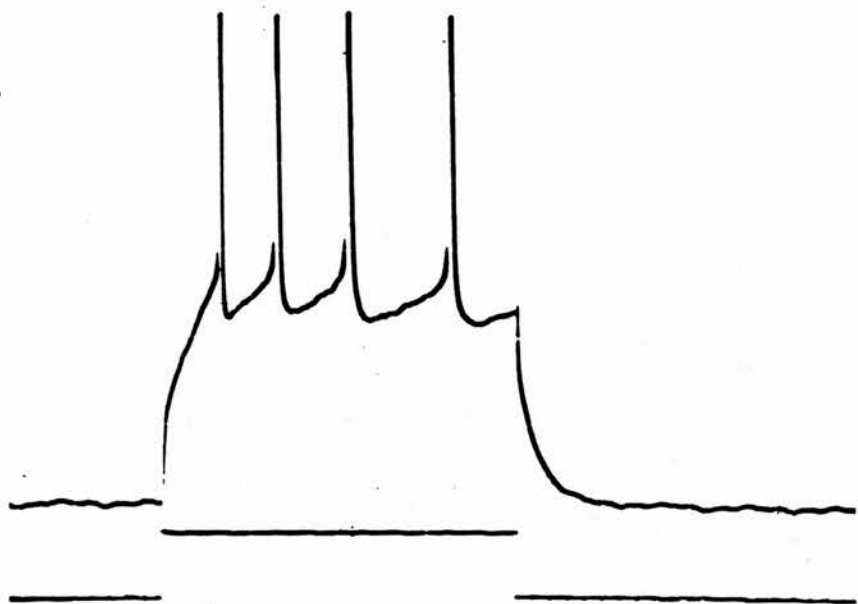
the appearance of rectification (Fig. 52). This occurred to a greater extent in the hyperpolarising direction, usually with currents greater than  $-1.2$  to  $-1.4$  nA. A further type of rectification was also observed with the passage of larger currents in the hyperpolarising direction, also with currents greater than  $-1.2$  to  $-1.4$  nA. This was observed as a sag in the original potential level after approximately 2ms to a lower, more stable level (Figure 51, arrow).

The input resistance of each neuron was calculated from the slope of the current voltage curves crossing at zero current point. Consequently, for the neuron used as an example in figure 52, an input resistance of 20M $\Omega$  was obtained, while the mean input resistance for a total of 28 neurons was  $21.79 \pm 4.7$  M $\Omega$ .

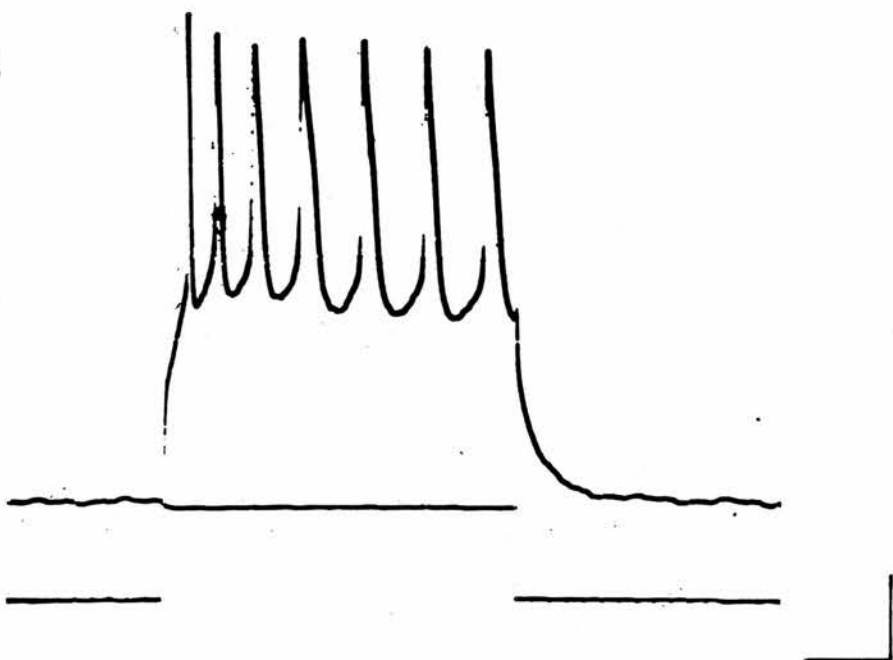
Tetraethylammonium (TEA) ( $5\mu$ M) was bath applied to five intracellularly recorded striatal neurons. It caused a decrease in resting membrane potential, and increased the duration of individual action potentials (Figure 53). It also reduced the amplitude of afterhyperpolarising potentials of individual spikes. Prolonged exposure led to the disappearance of action potentials altogether. For one striatal neuron, current-voltage relationships were plotted before and after the application of TEA (Figure 54). A decrease in rectification in both directions was observed. Neither prolonged exposure nor an increase in concentration of TEA applied up to  $20\mu$ M resulted in the appearance of prolonged plateau potentials.

Neurons ( $n = 3$ ) impaled with electrodes filled with 3M caesium chloride (CsCl) had very low resting potentials (approximately 40-50mV) and as a result were very unstable. However, short depolarising current pulses (8-10ms duration) gave rise to usually

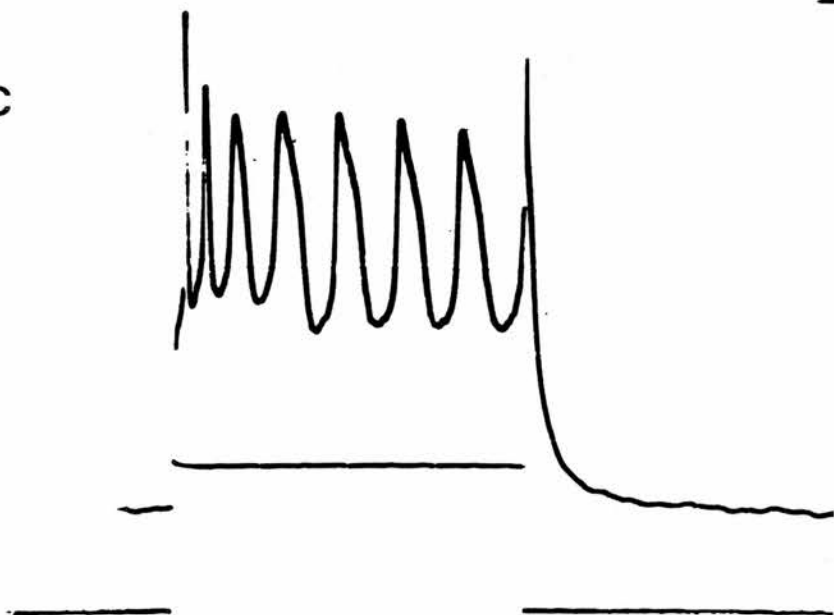
A



B



C



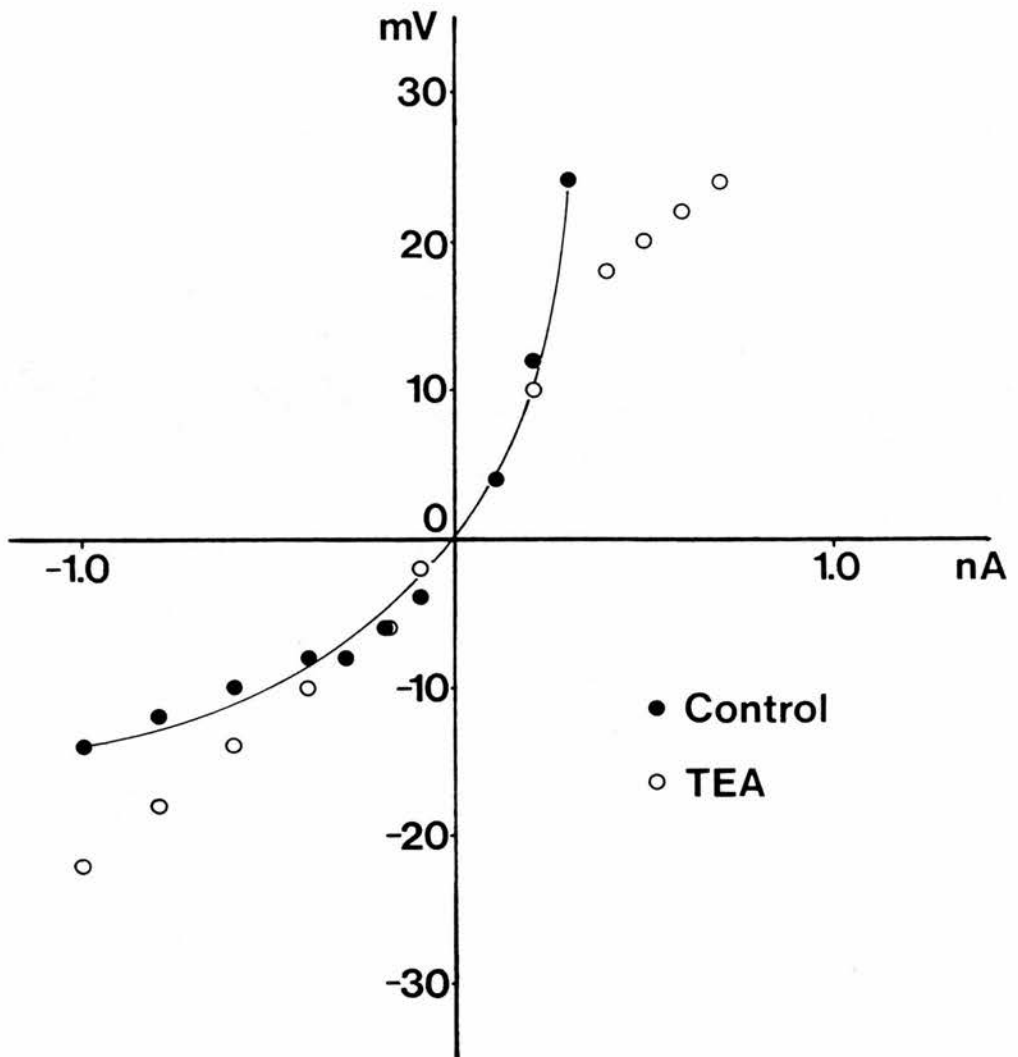


FIGURE 54. Current-voltage relationship of a striatal neuron before and after the bath application of TEA (10mM).

FIGURE 53. The effects of TEA on striatal action potentials. This neuron was stimulated intracellularly. Resting membrane potential = -80mV.  
 A. Control  
 B. 10 minutes after bath application of 10mM TEA.  
 C. 20 minutes after TEA.

The stimulus intensity was increased after TEA application in order to produce a clearer view of the effect.  
 Scale: 20ms, 20mV and 1nA.

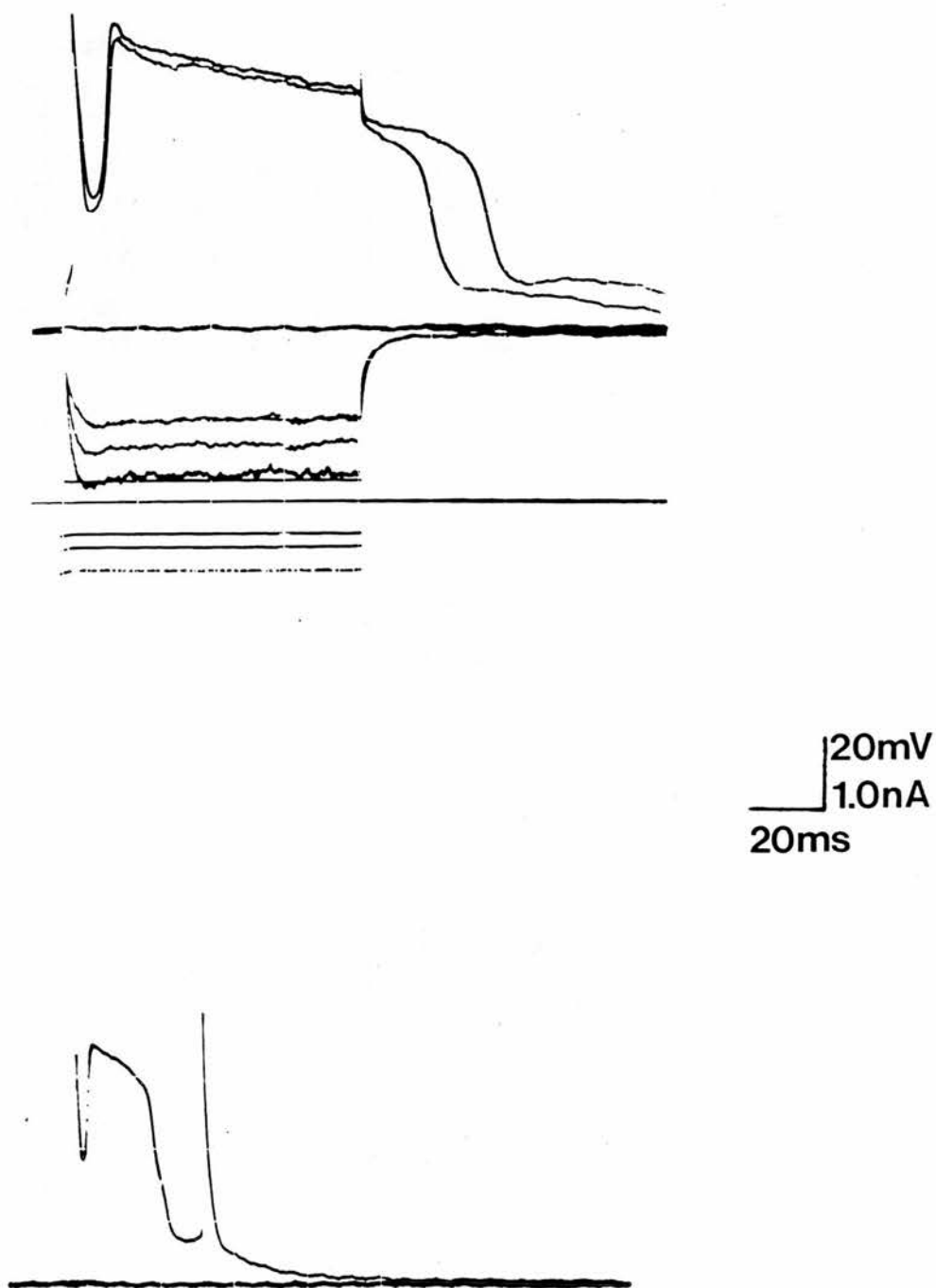


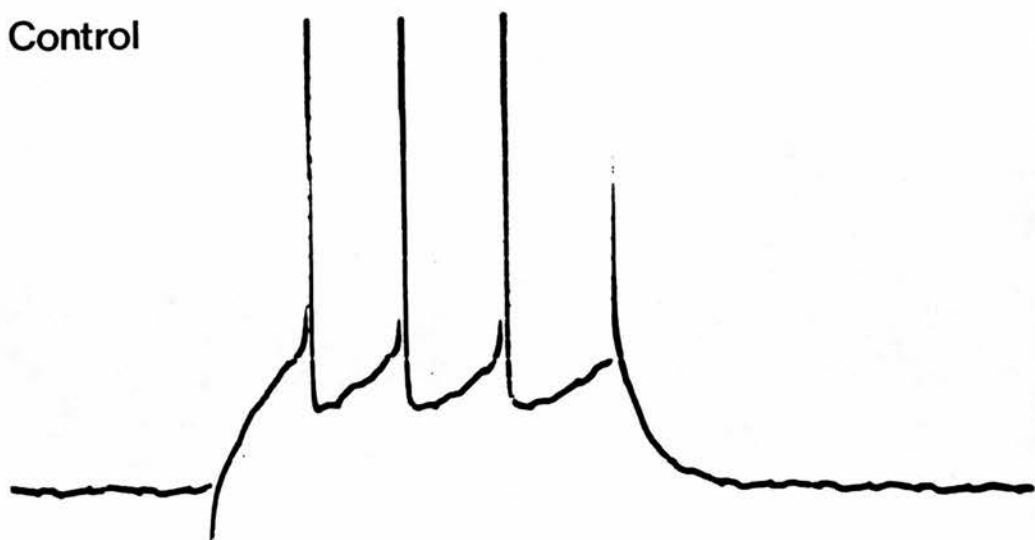
FIGURE 55. Examples of responses obtained from intracellularly recorded striatal neurons using caesium filled electrodes.  
A. Intracellular stimulation using depolarising and hyperpolarising current pulses.  
B. Cortical stimulation.

one fast-rising spike at the offset of the pulse which was often followed by a long-lasting plateau potential (duration 120ms or more) which outlasted the duration of the pulse. With wider pulses (80ms) a fast rising action potential was again observed during the pulse, followed by a prolonged plateau potential of similar duration to that observed with shorter pulses. In several instances the plateaux decayed rapidly towards baseline but before reaching that level were followed by a depolarising "hump" or the occasional wide action potential, after which the potential returned to zero level. The duration of this type of response was approximately 300msec. Activity in the hyperpolarising direction appeared relatively normal (Figure 55, top trace). Responses to cortical stimulation displayed similar activity when recorded with CsCl-filled electrodes (Figure 55, bottom trace).

Bath application of  $1\mu\text{M}$  TTX led to the abolition of all spike activity and synaptic potentials observed for three neurons (Figure 56). One neuron was held long enough to test the effects of TTX on the current-voltage relationship. It was found that the rectification usually observed in the outward direction (i.e. depolarising) was abolished, while the rectification in the hyperpolarising direction remained unchanged compared to that obtained in control medium for the same neuron (Figure 57).

Current-voltage curves were also constructed for 16 of these neurons after the application of DA. Figure 52 shows a typical effect of DA in that it produced a decrease of the rectification in the depolarising direction while having little effect on the shape of the plot in the hyperpolarising direction, nor on the hyperpolarising "sag" in these neurons. Similarly, no significant

Control



After TTX



FIGURE 56. The effect of TTX ( $1\mu\text{M}$ ) application on striatal neuron firing.  
Scale: 20ms, 20mV and 2nA.



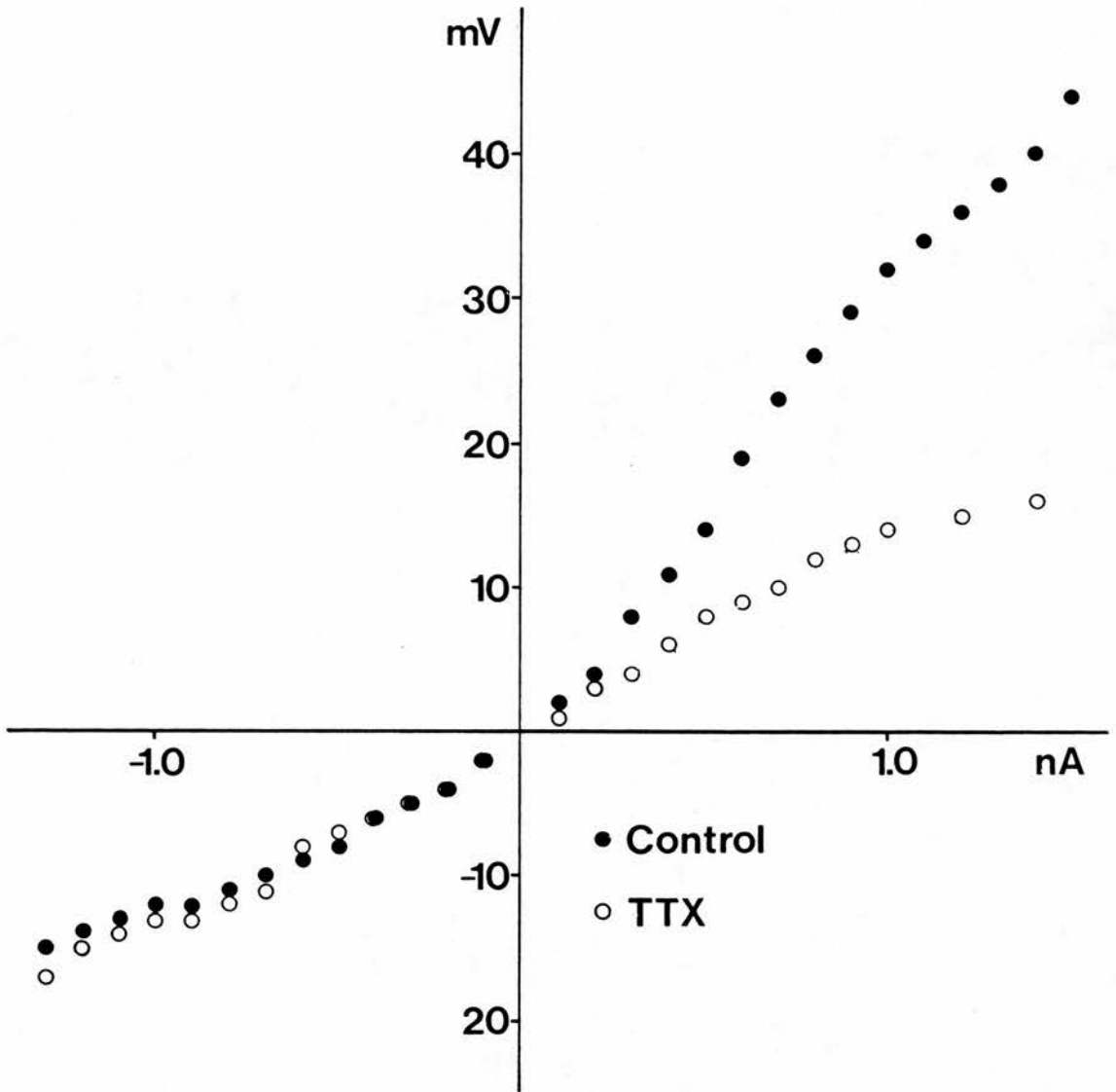


FIGURE 57. Current-voltage relationship of a striatal neuron before and after the bath application of TTX ( $1\mu\text{M}$ ).

change in the voltage threshold was apparent. As a result, the input resistance was not significantly different from the control values after DA application, the mean value being  $19.51 \pm 4.75$  M Ohms.

(iii) Firing frequency and accommodation

Increasing the intensity of a stimulus applied intracellularly resulted in an increase in the number of spikes elicited per stimulus (Figure 49). With most neurons a ceiling level of firing was achieved at sufficiently high current intensities but occasionally a failure of spike generation was observed. As a precaution, the intensity of stimulation was never taken too high as intracellular penetrations were often lost by the passage of large depolarising currents.

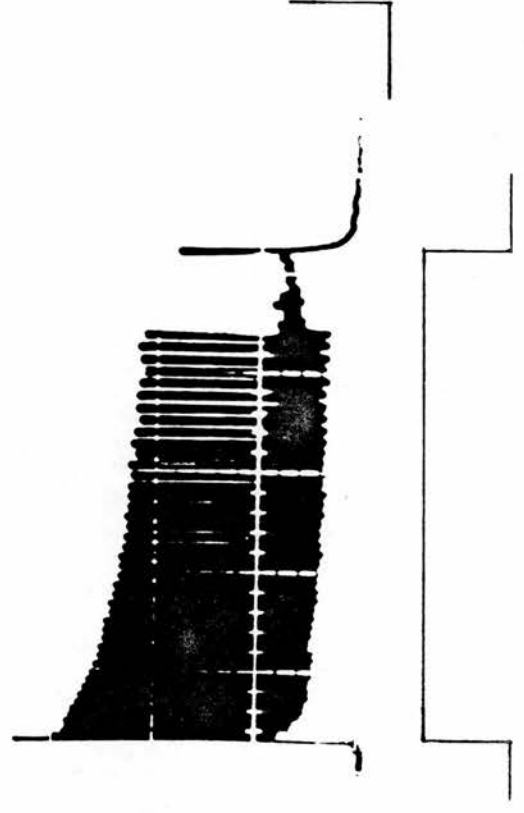
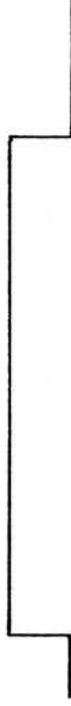
As demonstrated in section (ii), DA decreased the number of spikes evoked by depolarising pulses by increasing the threshold\* for spike activation. An increase in current restored the evoked action potentials which appeared unmodified.

Spike frequency accommodation was observed when short (80ms) and long (500ms) current pulses were applied to the neurons. This effect appeared more pronounced with longer pulses. Responses at 2x and 3x current threshold\* for spike activation were studied, although optimum accommodation was observed at 2x threshold (Figure 58). The rate of discharge was found to be highest at the beginning

\*Throughout this chapter the intensity of the depolarising pulse used was referred to the threshold current necessary to produce a single spike. Threshold was determined for each condition i.e. before and after DA or at every holding potential.

FIGURE 58. The effects of DA on accommodation of action potential discharge.  
Iontophoretically applied DA reduced accommodation as seen by an increase in the number of spikes produced by a depolarising pulse of 2x and 3x threshold.  
Scale: 100ms, 20mV and 4nA.

3x Threshold

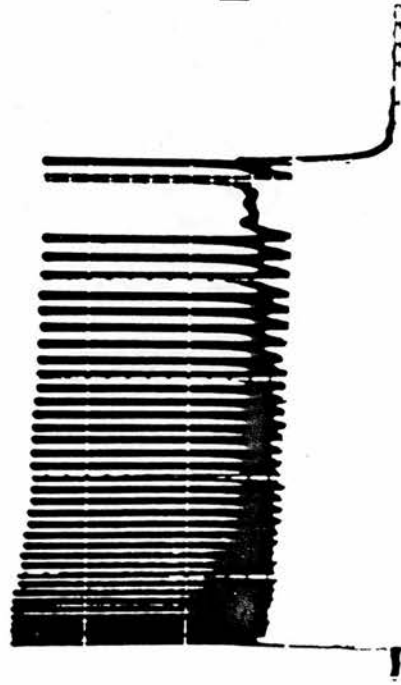


CONTROL

2x Threshold



DOPAMINE

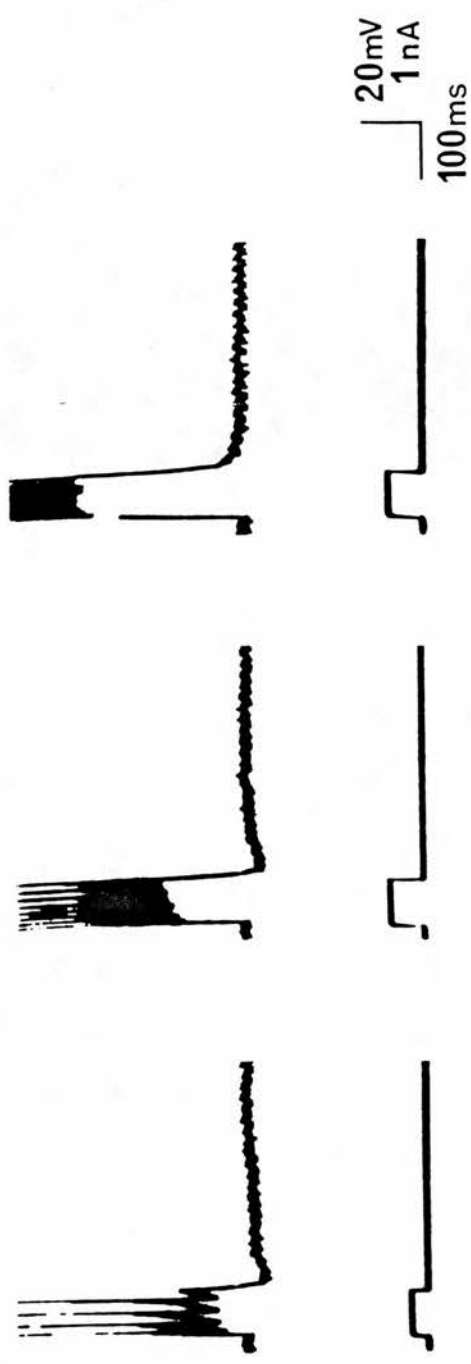


of the current application and gradually decreased with an accompanying decrease in spike amplitude (Figure 58). Occasionally, failure of spike generation was observed towards the end of the pulse. With higher current intensities (3x threshold) these phenomena became more accentuated with the result that spike failure occurred more quickly in all 9 cells in which it was tested. For 500ms wide depolarising pulses of intensity 2x threshold for spike activation the mean number of spikes elicited per pulse was  $30.3 \pm 9$  ( $n = 16$ ), while for stimuli of 3x threshold this value was  $39.91 \pm 12.6$  ( $n = 9$ ). After DA application equivalent 2x threshold depolarisations resulted in significantly more impulses (mean  $\pm$  SD,  $38 \pm 11$ ;  $p < 0.01$ , paired 't')(Figure 58). However, for equivalent 3x threshold depolarisations no significant difference in the number of spikes was observed (mean  $\pm$  SD;  $39.14 \pm 12.41$ ,  $n = 7$ ) presumably due to increased spike failure at such high levels of current.

(iv) Afterhyperpolarisation (AHP)

Under resting conditions a brief depolarising pulse (80ms wide, 2x threshold) evoked a train of action potentials with little or no AHP visible. However, as the neurons were depolarised by steady DC current injection to potentials between -70 and -55mV, further depolarisation evoked a single action potential or a train of spikes followed by a distinct AHP (Figure 59A). The AHP was usually absent at the more commonly observed resting potentials i.e. between -80 and -90mV, but if the neurons were hyperpolarised by steady DC current injections to below these levels, some evidence of reversal was occasionally observed, and was usually apparent at holding potentials between -89 and -95mV. An estimate of the reversal

A



B

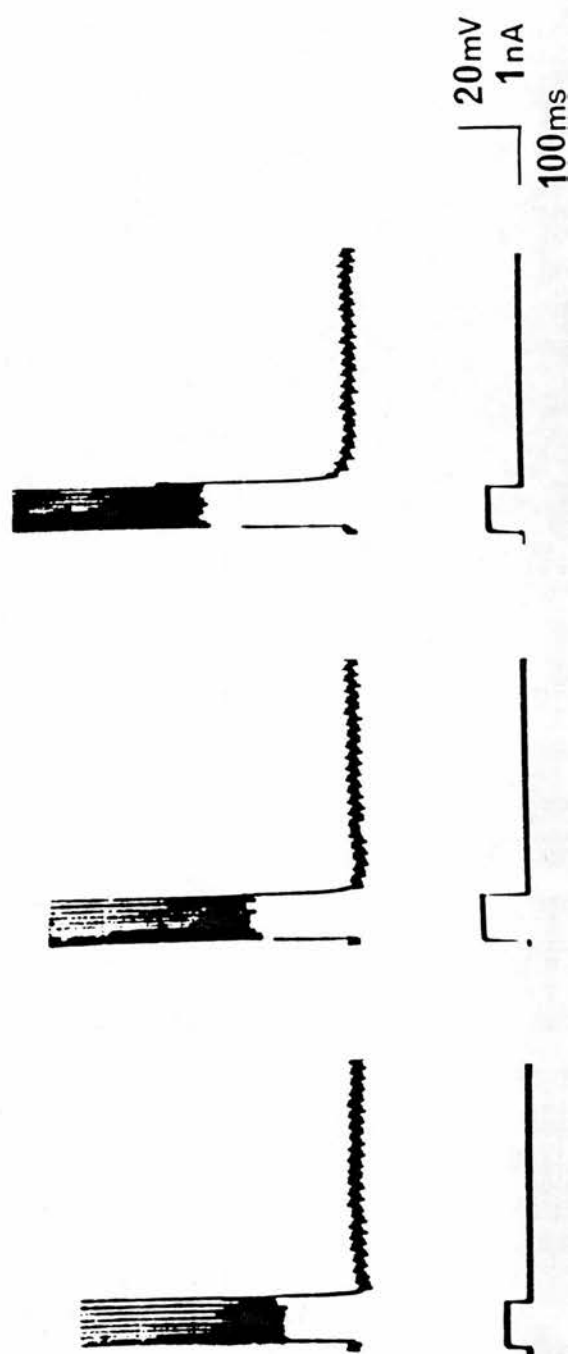


FIGURE 59. Changes in the amplitude of the AHP seen at different holding potentials. Intracellular current clamp records obtained from a striatal neuron stimulated at 2x threshold before (A) and after DA administration (B). The AHP amplitude decreases after DA application. The resting potential of this neuron was -85 mV.

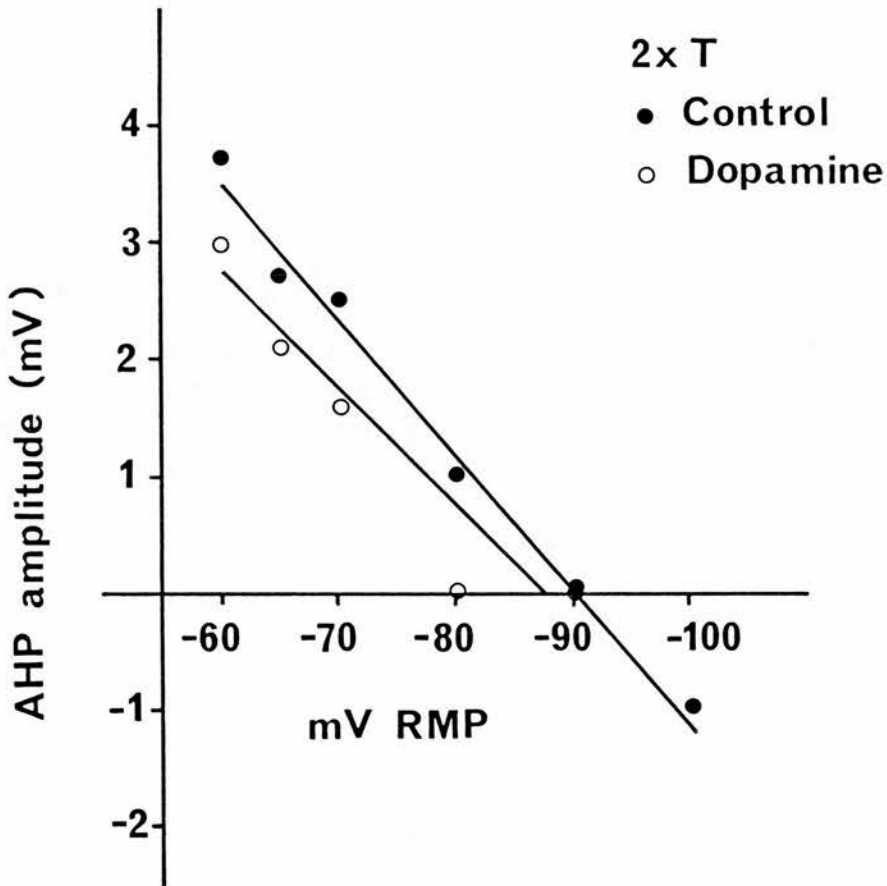


FIGURE 60. Graph of peak amplitude of AHP voltage versus membrane (or holding) potential at 2x threshold stimulating current. These measurements were made before and after DA administration. The reversal potential of this neuron is -90mV.

potential was also obtained by plotting the peak amplitude of the AHP as a function of the holding potential (Figure 60). This indicated that the reversal potential was  $-90\text{mV}$ .

At a fixed membrane potential of  $-70\text{mV}$  the AHP ranged from 1 to 5 mV in amplitude and 200–1000ms in duration depending on the stimulus parameters. As the duration of a threshold current pulse (mean  $\pm$  SD,  $0.42 \pm 0.2\text{mA}$ ;  $n = 12$ ) was increased from 80 to 800 ms, AHPs of progressively larger amplitude and longer duration were evoked. Similarly, when a depolarising current pulse of a fixed duration (80ms) and a variable intensity was used, it was also found that the AHP increased in amplitude with increasing depolarisation. This was demonstrated by fixing the membrane potential to  $-70\text{mV}$  and changing the intensity of stimulation (Figure 61) and was also found to occur at still lower holding potentials i.e.  $-65\text{mV}$  and  $-60\text{mV}$ .

DA reduced the amplitude of the AHP as seen from the shift in the graph of AHP amplitude at different membrane potentials (Figures 59 and 60) and with various current intensities (Figure 61). On the 45 occasions when an AHP was elicited by equivalent stimuli at identical membrane potentials in the same neuron before and after DA application, the reduction was highly significant ( $p < 0.01$ ; paired 't' test).

It was apparent that the application of  $1\mu\text{M}$  TTX abolished the AHP observed in these neurons. However, it could be debated that this was because the threshold for  $\text{Ca}^{++}$  action potentials was not reached in these experiments, and so the associated  $\text{Ca}^{++}$  influx did not occur and the AHP was thus not triggered.

The AHP was also substantially reduced in one experiment after bath application of a medium in which calcium was replaced by an

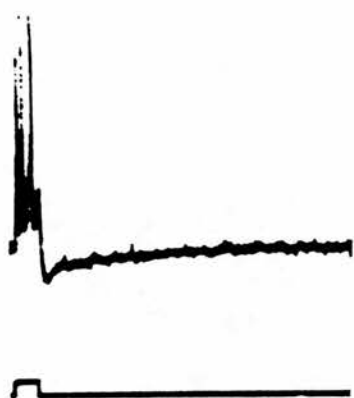


A

-240-

2 x T

3 x T



20mV  
1nA  
200ms

B

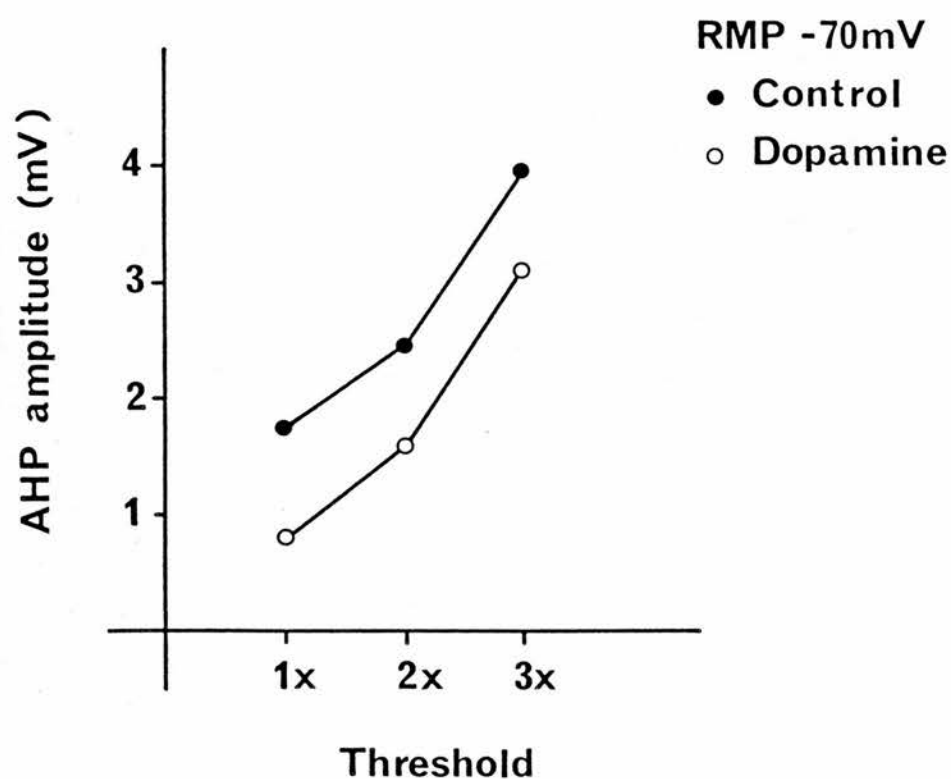
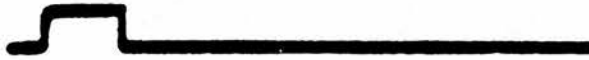
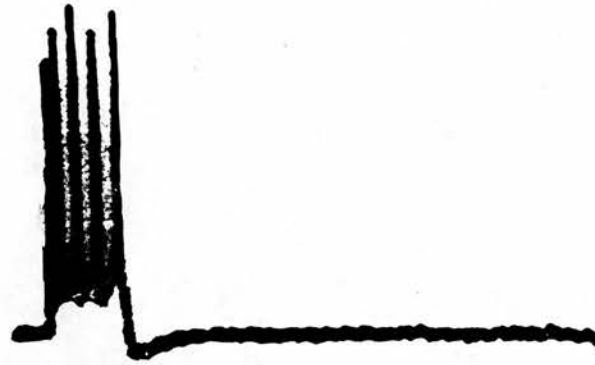


FIGURE 61. Changes in the amplitude of the AHP at a fixed resting potential (RMP) of -70mV seen at different intensities of stimulation with a depolarising current pulse (80ms wide).

- A. Representative intracellular current clamp records at 2x and 3x threshold (T) for spike activation.
- B. Graph of peak amplitude of AHP voltage versus different intensities of stimulation. Application of DA reduces the AHP amplitude at each current level tested (open circles).

Control



Ca<sup>2+</sup> Free

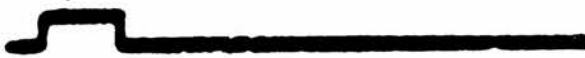


FIGURE 62. The effects of Ca<sup>2+</sup>-free medium on the amplitude of the AHP at a fixed holding potential of -65mV and a fixed current intensity of 2x threshold for spike activation (80ms wide)  
Scale: 20ms, 20mV and 2nA.

# Reversal Potentials

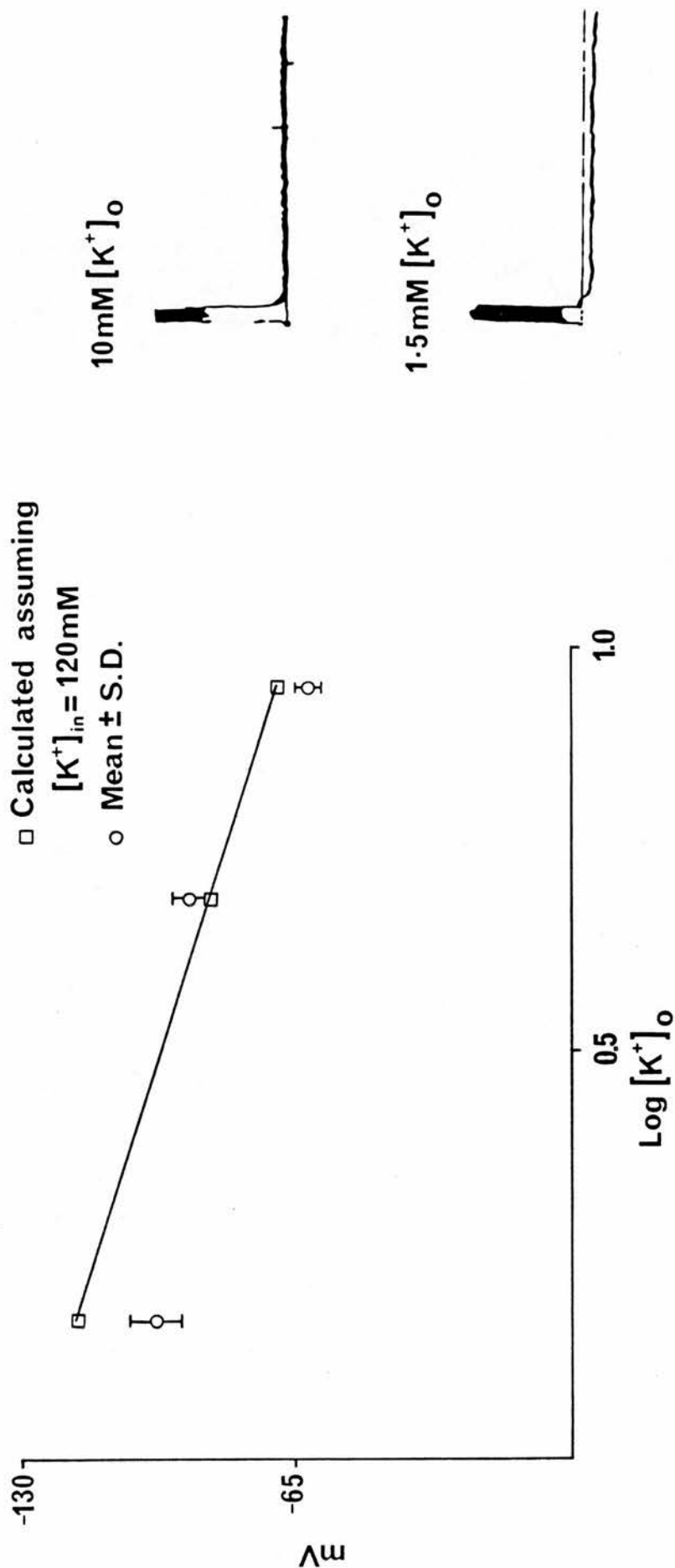


FIGURE 63. Graph of reversal potentials observed using different potassium concentrations. The reversal potential of the AHP was determined using medium containing 1.5mM, 4.5mM and 10mM potassium. These values are compared to those calculated assuming  $[\text{K}]_{in} = 120 \text{ mM}$ . Examples of reversal potentials at 4.5mM and 10mM potassium are also shown.

equimolar concentration of magnesium (2mM)(Figure 62). In this and two other experiments, the amplitudes of individual spike AHPs were also reduced substantially.

It was found that an alteration of the extracellular  $K^+$  concentration led to a change in the AHP reversal potentials such that 1.5mM  $K^+$  made the AHP reverse at more hyperpolarised potentials, while 9.5mM  $K^+$  had the reverse effect. Figure 63 shows the similarities between these values and those calculated from the Nernst equation assuming that internal  $K^+$  concentration is 120mM. A low external  $K^+$  concentration also led to other afterpotentials, which may be related to the plateau potentials seen in caesium-filled neurons and which made determination of the reversal potential very difficult. On the other hand some neurons were spontaneously active in medium with higher  $K^+$  concentration and this may have influenced the results.

#### Responses to cortical stimulation

Stimulation of the cortex whilst recording intracellularly within the striatum evoked depolarising potentials which appeared similar to the EPSPs observed in other 'in vitro' (Calabresi et al., 1987; Calabresi, Misgeld and Doht, 1987) and 'in vivo' studies (Mercuri et al., 1985) (Figure 64A). In all neurons studied, no IPSPs were observed to follow these EPSPs. The EPSP latencies were short (mean  $\pm$  SD,  $6.33 \pm 2.26$ ms),  $n = 40$ ) and the mean current threshold for EPSP activation was  $0.4 \pm 0.35$ mA ( $n = 40$ ). The latency of the EPSP remained unchanged after varying the stimulus strength and also after 3 pulses delivered at high frequency (800Hz) (Figure 64B) which suggested that the response observed was monosynaptic.

FIGURE 64. Intracellular recordings of three striatal neurons responding to cortical stimulation.

A. Single EPSP

B. The effect of high frequency stimulation on the EPSP latency.

C. An increase in the stimulus intensity leads to an increase in amplitude of the EPSP and eventually an action potential.

As the stimulus intensity is increased the duration of the long-lasting depolarisation also increases.

Scale: 20ms, 20mV.

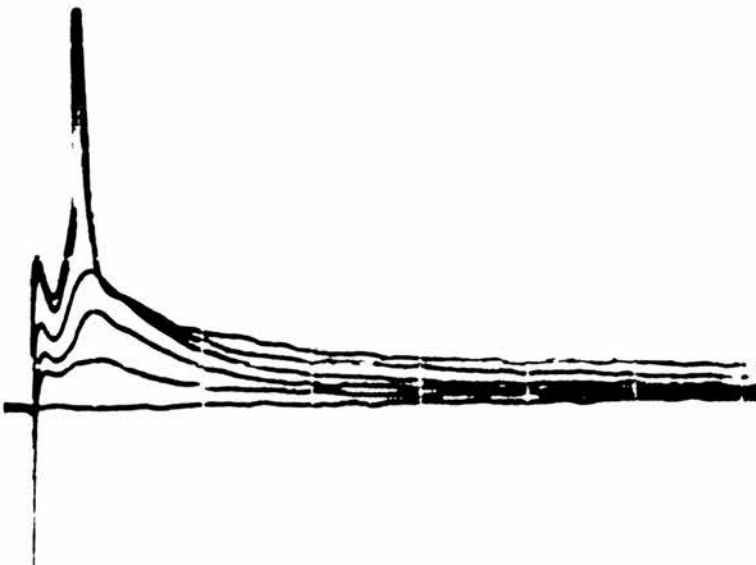
A



B



C



An increase in stimulus strength gave rise to a concomitant increase in EPSP amplitude such that at sufficient levels of applied current, single action potentials were seen to arise from the EPSPs (Figure 64C). Not all EPSPs gave rise to an action potential, even at maximum stimulation. The mean latency of the evoked action potential was  $5.68 \pm 5.03\text{ms}$  ( $n = 38$ ) while mean threshold for spike activation was  $0.99 \pm 0.44\text{mA}$  ( $n = 38$ ).

The duration of the EPSPs usually ranged from 10–20ms (Fig. 64A). At levels of stimulation sufficient to evoke action potentials, long lasting depolarisations (100–250ms duration) were also observed, this phenomenon being more clearly seen when the intensity of cortical stimulation was relatively strong (Figure 64C).

DA was applied via the bath ( $0.5\mu\text{M}$  or  $1\mu\text{M}$ ) or iontophoretically ( $25\text{--}50\text{nA}$ , 3–5min) to 17 out of the 40 neurons displaying a response to cortical stimulation. The effects observed were the same regardless of concentration applied or method used; in all cases the cortical response was inhibited by DA such that 21% of the neurons tested no longer yielded action potentials even at maximum stimulation. Those neurons in which action potentials could still be evoked displayed significantly higher thresholds for spike activation ( $p < 0.05$ , paired 't' test) although the thresholds for EPSP activation were not significantly different. No detectable differences between EPSP amplitudes were observed although at threshold these amplitudes were so small that any changes would be difficult to observe. Similarly, latency and duration of each response remained unchanged, but the duration of the long-lasting depolarisation was significantly increased ( $p < 0.05$ , paired 't' test)(See Table 9).

TABLE 9. Cortical stimulation: Values of various parameters before and after the application of DA.

PARAMETER		CONTROL	AFTER DA
EPSP	Threshold(mA)	$0.4 \pm 0.35$ (n = 40)	$0.32 \pm 0.24$ (n = 17)*
	Duration(ms)	$18.38 \pm 4.16$ (n = 24)	$19.3 \pm 3.06$ (n = 10)
	Latency(ms)	$6.33 \pm 2.26$ (n = 40)	$6.41 \pm 2.22$ (n = 17)
Spike	Threshold(mA)	$0.99 \pm 0.44$ (n = 38)	$1.13 \pm 0.42$ (n = 14)**
	Latency(ms)	$5.68 \pm 5.03$ (n = 38)	$4.92 \pm 2.14$ (n = 13)
Long-lasting depolarisation	Duration(ms)	$180 \pm 60$ (n = 6)	$327 \pm 155$ (n = 6)**

\* p > 0.05

\*\* p < 0.05



## DISCUSSION

### Membrane properties

A major finding of the present study was that impaled striatal neurons had, on average, a much more negative resting potential than those reported in other 'in vitro' and 'in vivo' studies (c.f. Kita et al., 1984; Calabresi et al., 1987). Unlike other studies, neurons were discarded if their resting potentials were more positive than  $-65\text{mV}$  as it was generally found that only short-lived recordings of these neurons were possible.

One explanation for the discrepancies in resting potentials is clear if a comparison of the constituents of the bathing medium is made (Table 10). Most other studies made use of a higher concentration of potassium, which is often more than  $6\text{mM}$  compared to the more physiological concentration of  $4.5\text{mM}$  (CSF,  $3.3\text{mM}$ ; Plasma,  $5.0\text{mM}$ ) used here. This would tend to depolarise the neuron somewhat and consequently increase the incidence of spontaneous activity. The complete lack of spontaneous activity observed in this study is in direct contrast to that of Akaike et al (1987), who, with a potassium concentration of  $5.4\text{mM}$  and some resting potentials as low as  $-55\text{mV}$ , observed spontaneous activity in approximately 13% of the neurons tested.

Application of DA had no significant effect on the membrane potential of any of the neurons tested, regardless of whether it was added to the bath or applied by iontophoresis. This result is in agreement with that obtained by Calabresi et al (1987) who bath applied similar concentrations of DA as those used here. On the other hand however, Akaike et al (1987) found that a low

TABLE 10 : COMPARISON OF MEDIA AND CONDITIONS USED BY DIFFERENT INVESTIGATORS

	NICOLL	NORTH	PRINCE	KITAI	LLINAS	CHERUBINI	CALABRESI	RUTHERFORD
NaCl	116.4	126	124	124	124	126	125	124
KCl	5.4	2.5	5	5	5	3.5	5	3.3
CaCl <sub>2</sub>	2.5	2.4	2	2	2.4	2	2.5	2.5
MgSO <sub>4</sub>	1.3		2	2	1.3		1.3	2.4
MgCl <sub>2</sub>		1.3				1.2		
NaHCO <sub>3</sub>	26.2	26	26	26	26	25	25.5	26
NaH <sub>2</sub> PO <sub>4</sub>	0.92	1.2	1.25			1.2		
KH <sub>2</sub> PO <sub>4</sub>				1.25	1.2		1.2	1.25
glucose	11	10	10	10	10	10	10	10
species	rat , guinea pig	rat	guinea pig	rat , guinea pig	guinea pig	rat	rat	rat
temperature(°C)	30-32	36	37.5	37	37	room	36	36

concentration of DA ( $1\mu\text{M}$ ) produced a depolarisation of approximately 5mV while larger concentrations ( $100\text{--}500\mu\text{M}$ ) had no effect.

Intracellular recordings carried out 'in vivo' are equally contradictory (e.g. Mercuri et al., 1985; Herrling and Hull, 1980).

Studies carried out in the hippocampus (Gribkoffe and Ashe, 1984; Benardo and Prince, 1982a; Stanzione et al., 1984) and nucleus accumbens (Uchimura et al., 1986) have also given rise to some confusion regarding the effects of DA on membrane potential. In contrast to this, both 'in vivo' and 'in vitro' examination of cortical neurons (Bernardi et al., 1982; Penit-Soria et al., 1987) have observed a depolarising effect of DA, while in SN zona compacta neurons the effect observed is predominantly a hyperpolarisation (Grace and Bunney, 1985; Lacey et al., 1986; Pinnock, 1983).

The mean value obtained for input resistance was similar to that obtained from rat striatum 'in vitro' (Misgeld et al., 1982; Kita et al., 1984) and cat striatal neurons 'in vivo' (Sugimori et al., 1978). These values were also similar to those obtained for other medium-sized neurons in the thalamus (Kita et al., 1983) and inferior olive (Llinas and Yarom, 1981).

Rectification, seen as a decrease in input resistance, was observed when the neuronal membrane was hyperpolarised by current injections in all neurons examined. This was in agreement with other 'in vivo' (Sugimori et al., 1978) striatal studies. It has also been demonstrated in SN (Kita et al., 1986), thalamus (Jahnsen and Llinas, 1984) and cortex (Stafstrom et al., 1984).

In addition to this inward rectification was the frequent observation of a time dependent "sag" of hyperpolarising potentials following an initial overshoot and which was present with more

extreme levels of hyperpolarising current (i.e. greater than  $-1.2\text{nA}$ ). This phenomenon has been reported in other central neurons 'in vitro' (Henderson et al., 1982; Constanti and Galvan, 1983; Kita et al., 1986) and 'in vivo' (Purpura et al., 1968; Takahashi, 1965; Nelson and Frank, 1967). Kita et al (1986), using a SN slice preparation, reported that the inward rectification as well as the "sag" could be largely reduced with the addition of TEA to the bathing medium. This was confirmed by preliminary experiments carried out in the present study.

During voltage-clamp experiments on guinea pig olfactory cortex neurons 'in vitro', Constanti and Galvan (1983) found that this rectification was activated between  $-100$  and  $-110\text{mV}$  and was unaffected by TTX or cadmium. They proposed that it was due to a fast inwardly rectifying potassium current by virtue of its dependency on external potassium concentration and its blockade by caesium and barium. The application of TEA resulted in an effect similar to that of lowering external potassium (c.f. slow inward rectifier ( $I_Q$ ) of Halliwell and Adams, 1982; Lacey and North, 1988).

Rectification following subthreshold depolarising current pulses was also observed in the present study. This confirms the findings of previous investigations in striatum (Kita et al., 1984; Calabresi et al., 1987; Sugimori et al., 1978) hippocampus (Hotson et al., 1979; Brown and Griffith, 1983; Benardo and Prince, 1982; Stanzione et al., 1984; Johnston et al., 1980), cortex (Stafstrom et al., 1982; Galvan et al., 1982), thalamus (Jahnsen and Llinas, 1984) Purkinje cells (Llinas and Sugimori, 1980) and also invertebrate neurons (Smith et al., 1975; Eckert and Lux, 1976).

The exact ionic nature of this rectification seems to be somewhat controversial. Several studies report that while the rectification is reduced by TTX, it is not greatly affected by the blockade of calcium conductances. This has led to the speculation that a persistent sodium conductance is responsible for the rectification (Calabresi et al., 1987; Connors et al., 1982; Jahnsen, 1986; Llinas and Sugimori 1980; MacVicar, 1985; Stafstrom et al., 1982; 1985). On the other hand, there is also evidence to suggest that in some cells it is affected by the manipulation of calcium concentration but not by TTX (Brown and Griffith, 1983; Galvan et al., 1985) while other studies have implied that both sodium and calcium conductances are involved (Hotson et al., 1979; Smith et al., 1975; Eckert and Lux, 1976; Benardo and Prince, 1982b).

The present study confirmed the action of TTX, but the effects of calcium blockers or calcium-free medium on the rectification were not examined. However, preliminary experiments suggest that TEA reduces the rectification in both directions, implying that a potassium conductance may also be involved. Further investigations are required to elucidate the nature of the rectification in striatal neurons.

#### The effects of DA on firing frequency

The present investigation confirms previous studies carried out 'in vivo' and 'in vitro' which reported an inhibitory action of DA on striatal neurons (Bernardi et al., 1978; Herrling and Hull, 1980; Mercuri et al., 1985; Calabresi et al., 1987). As demonstrated in both striatum and other central neurons (Stanzione et al., 1984; Calabresi et al., 1987) DA decreased the frequency of current-evoked

action potentials by increasing the threshold for spike activation, an action which was coupled with a decrease of the depolarisation-induced rectification in the absence of changes in resting potential.

It can be suggested therefore that DA decreases spike firing not through changes in membrane potential but by affecting the ionic currents underlying the regulation of the spike generating mechanism. This hypothesis is supported by the demonstration that an increase of the injecting current restored the spikes evoked by depolarising pulses but abolished by DA, thus effectively ruling out any possibility that the block of the spikes was related to action potential inactivation.

The DA-induced changes of the current-voltage relationships suggested that it affects excitability in the subthreshold depolarised voltage range by depressing a conductance underlying the depolarisation induced rectification. Calabresi et al (1987) indicate that it is a TTX-sensitive conductance, such as a persistent inward sodium conductance which has been described in other neurons (Stafstrom et al., 1982, 1985; Connors et al., 1982; Jahnsen, 1986; Llinas and Sugimori 1980; MacVicar, 1985).

Several other investigations carried out on central neurons have indicated that the transient outward potassium current,  $I_a$ , might be involved in the regulation of action potential activation in a similar manner (Segal and Barker, 1984; Zbicz and Weight, 1985; Colino and Halliwell, 1987). However, Calabresi et al (1987) suggested that the reported characteristics of this current implies that in striatum, subthreshold voltage steps would not activate it at rest, although this does not exclude the involvement of other potassium currents.

### Accommodation and the AHP

A characteristic feature of many neurons is that they accommodate their responses to impinging stimuli (Madison and Nicoll, 1984; Constanti and Sim, 1987; Kita et al., 1986; Bourque et al, 1985). The present study, like that of Kita et al (1985a), confirms that striatal neurons are no exception; the responses of recorded neurons to a depolarising stimulus consisted of an initial rapid train of action potentials which slowed towards the end of the stimulus and was on occasions seen to stop altogether. Concomitant with the ability to accommodate was the presence of a distinct AHP following the repetitive firing induced by long depolarising current injections, the degree of AHP being related to the intensity of the evoked firing.

A similarly slow AHP has also been observed in other CNS neurons (Kita et al., 1986; Connors et al., 1982; Stafstrom et al., 1984; Higashi et al, 1987; Bourque et al., 1985; Jahnsen and Llinas, 1984; Constanti and Sim, 1987), although its characterisation has been carried out to a greater extent in the hippocampus (Thompson et al., 1985; Lancaster and Nicoll, 1987; Lancaster and Adams, 1986; Gustafsson and Wigstrom, 1981; Madison and Nicoll, 1984; Storm, 1987; Wong and Prince, 1981; Alger and Nicoll, 1980; Colino and Halliwell, 1987). These AHPs have been attributed to the activation of a calcium dependent potassium conductance and can be blocked by the removal of external calcium or by the addition to the bathing medium of divalent cations (e.g.  $Mn^{2+}$ ,  $Ca^{2+}$ ,  $Co^{2+}$ ) which interfere with calcium entry (Wong and Prince, 1981; Madison and Nicoll, 1984; Malenka and Nicoll, 1986; Constanti and Sim, 1987;



Lancaster and Nicoll, 1987). Preliminary experiments carried out in the present study have confirmed that the removal of external calcium reduces AHP amplitude although the use of calcium-blockers for this purpose was not attempted.

The amplitude and duration of the AHP observed under current clamp in striatal neurons are slightly smaller than those observed in hippocampal or cortical neurons. A study carried out by Thompson et al (1985) demonstrated that amplitude and duration of the AHP were temperature-dependent such that lower temperatures gave rise to larger amplitudes and durations. As the present experiments were carried out at 36-37°C, this seemed the most likely explanation for the small amplitude AHPs obtained.

As noted previously (Benardo and Prince, 1982a, 1982b) the reversal of the AHP was difficult to obtain with normal  $K^+$  concentration but by plotting the amplitude of the AHP versus the holding potential, the reversal potential estimated was similar to that of neurons from other brain areas demonstrating a slow AHP (Benardo and Prince, 1982b; Constanti and Sim, 1987). In addition, the observation that the reversal potential was altered by changing the concentration of the potassium in the bathing medium further confirms the involvement of a potassium conductance in the AHP.

The AHP in the present study, like that reported by Constanti and Sim (1987) in cortical neurons, appeared to be sensitive to TTX implying that its generation was somehow linked to neuronal sodium entry. Thus the inhibition of the AHP by TTX could arguably be due to suppression of the action potentials elicited by a depolarising pulse, and the consequent reduction in calcium entry. Other studies have demonstrated that the AHP does persist in the presence of TTX



but only when currents large enough to evoke TTX-resistant action potentials were used (Hotson and Prince, 1980; Gustafsson and Wigstrom, 1981). TTX-resistant action potentials have been reported in striatal neurons and are presumed to be mediated by calcium (Kita et al., 1985b; Cherubini and Lanfumey, 1987; Calabresi, Misgeld and Doht, 1987). The blockade of the AHP by TTX in the present experiment was seen only with current pulses insufficient to elicit calcium spikes; larger currents were not applied.

#### The effects of DA on the AHP and accommodation

Up until the present study, there have been no reports in the literature concerning the effects of DA on the striatal AHP. Rather, all of the investigations have been carried out in the hippocampus, which at first is somewhat surprising as the hippocampus is not considered to be a major part of any dopaminergic system. However, because of the rapid success in recording from hippocampal slices and also that there is some evidence of a mesencephalic DA-containing projection (Swanson, 1982), the hippocampus has been used to some extent as a model system in which to examine the postsynaptic actions of DA.

Benardo and Prince (1982a) found that DA augmented the AHP following spike trains in hippocampal neurons and suggested that these effects were mediated by an increase in intracellular calcium concentration resulting in the activation of the calcium-dependent potassium conductance which underlies the AHP. On the other hand, Stanzione et al (1984) reported that DA attenuated calcium-mediated events such as bursting activity and the AHP. Since then, other contradictory studies have followed (Pockett, 1985; Malenka and Nicoll, 1986; Gribkoff and Ashe, 1984).

The findings of the present study confirm those of Stanzione et al (1984) and Malenka and Nicoll (1986) in that DA reduces the amplitude and duration of the AHP following repetitive firing. A decrease in the accommodation of spike firing was also observed. Similar effects were recorded with serotonin (Colino and Halliwell, 1987) and NA (Madison and Nicoll, 1981; Lancaster and Nicoll, 1987; Storm, 1987) in the hippocampus and also after the application of NA on to cortical neurons (Constanti and Sim, 1987). NA was shown to eliminate the AHP and modify spike frequency accommodation by acting on  $\beta$ -adrenergic receptors (Lancaster and Nicoll, 1987). This action is mimicked by 8-bromo-cyclic AMP, an analogue of cyclic AMP; the effect is thus thought to be mediated via a second messenger system.

Malenka and Nicoll (1986) presented evidence to suggest that DA mimicked the effects of NA on the AHP by way of cross-reactivity with  $\beta$ -adrenergic receptors since  $\beta$ -adrenergic antagonists completely blocked the DA inhibition. Stanzione et al (1984) on the other hand, found that phentolamine (a  $\beta$ -adrenergic antagonist) had no effect on the actions of DA while domperidone blocked the DA inhibition of the AHP.

The inhibitory effect of DA in striatum has been attributed to the action of D1 dopaminergic receptors and the subsequent stimulation of cyclic AMP (Calabresi et al., 1987; Akaike et al., 1987). It is possible therefore that the action of DA on the AHP may also be mediated by D1 receptors. Since the present study does not provide any direct evidence to support this suggestion, this requires further investigation by the use of specific D1 and D2 receptor agonists and antagonists.

Muscarine has also been found to elicit a reduction of the AHP, which seems to be independent of cyclic AMP levels (Lancaster and Nicoll, 1987; Constanti and Sim, 1987). It is possible that DA-sensitive neurons in striatum may also possess muscarinic receptors (Nastuk and Graybiel 1985) but the action of acetylcholine on the AHP of these neurons remains to be explored.

#### Mechanism underlying the DA effect on the AHP

The exact ionic mechanism by which DA affects the AHP remains somewhat of a mystery. Stanzione et al (1984) proposed that a possible action of DA is in the reduction of calcium currents, an action of DA which has been demonstrated in burst firing cells in Aplysia californica (Gospe and Wilson, 1980). In that, and the present study, DA inhibited the AHP regardless of firing rate which suggests that this effect was not evoked via an action on the firing rate but that it seems to be related to a reduction of the calcium influx and/or to an interference of DA with the coupling between potassium and calcium currents. In support of an effect on the calcium influx, a decrease of the voltage-dependent calcium influx induced by DA has been observed in central neurons (Bossu and Feltz, 1981; Douglas and Tavaskevich, 1982; Stanzione et al., 1984). However, conclusive data concerning the membrane currents involved in the action of DA await investigation by voltage clamp studies.

#### Responses to cortical stimulation

In Chapters 3 and 4 where extracellular recording was carried out, cortical stimulation was used to search for normally silent striatal neurons. Here, direct stimulation was used to impale

neurons and so there was no guarantee that they would respond to cortical stimulation.

Stimulation of the cortex evoked depolarising potentials in some striatal neurons which appeared very similar to EPSPs observed 'in vivo' (Herrling and Hull, 1980; Mercier et al., 1985; Vandermaelen and Kitai, 1980; Herrling, 1985; Buchwald et al., 1973; Hull et al., 1973), and locally evoked EPSPs in 'in vitro' experiments (Calabresi et al., 1987; Calabresi, Misgeld and Doht, 1987; Kitai et al., 1984; 1985a; 1985b). However, this study is the first one to demonstrate a cortically evoked EPSP 'in vitro'; the one other documented study in which a similar type of preparation was used only examined extra-cellular recordings (Miller, 1981). An argument in favour of the present responses being cortically- rather than locally-evoked has been discussed in Chapter 3. It is likely that some of the locally-evoked responses observed in other studies are due to stimulation of corticostriatal afferent fibres severed from their cell bodies, since their pharmacology suggests that local responses are mediated by glutamate receptors with an additional NMDA component (Herrling, 1984).

No IPSPs were observed as such in this study, which is in direct contrast to experiments carried out 'in vivo' (e.g. Herrling and Hull, 1980; Mercuri et al., 1985) or 'in vitro' (Lighthall et al., 1981; Lighthall and Kitai, 1983), with slices from young (15 day old) animals (Misgeld et al., 1986) or in those exposed to barbiturates (Misgeld et al., 1982). In confirmation of the findings of Calabresi et al (1987) and Kitai et al (1984) no direct evidence of an IPSP was found here. Kitai et al (1984) suggested that the EPSPs may be compounded with IPSPs. This

explanation would be a distinct possibility as inhibition was observed extracellularly in Chapters 3 and 4, implying that an inhibitory mechanism was maintained within the slice.

The effects of DA on the cortically evoked EPSP were inhibitory. In no case was the membrane potential altered, rather DA appeared to exert its depressant action on the responses by increasing the amount of current required to elicit an EPSP. In most cases the threshold for spike activation was also increased, or the activation of a spike was blocked completely. Latency and duration of the EPSPs remained unchanged. This was in contrast to the study of Calabresi et al (1987) who found that EPSP duration was decreased. Experiments on striatum (Kita et al., 1984; Calabresi et al., 1987) and cortex (Stafstrom et al., 1982) 'in vitro' showed that the amplitude of the EPSP could be decreased by hyperpolarising the membrane (although Mercuri et al.(1985) found this treatment increased the EPSP amplitude while decreasing that of the IPSP). Calabresi et al (1987) found that while DA decreased the amplitude and duration of the EPSP at resting and depolarised levels of potential, it did not affect these parameters at hyperpolarised levels. Their mean value for resting potential was  $-69 \pm 4\text{mV}$  whereas the resting potentials obtained in this study were often at least 15mV more hyperpolarised. This therefore might account for the fact that changes in amplitude or duration were not observed.

The evidence presented by Calabresi et al (1987) to suggest that DA does not affect the amplitude or duration of the EPSP at hyperpolarised levels implies a voltage-dependent modulation of the EPSP by DA, which is activated at depolarised membrane potentials. A slow inward persistent sodium (Na) current was described in cat

cortical neurons by Stafstrom et al (1985) which was thought to increase the amplitude of both directly- and synaptically-evoked depolarisations. Kita et al (1984) on the other hand, described a strong decrease of the synaptic potential during membrane hyperpolarisation and related this finding to the rectification of the membrane in the hyperpolarising direction. From the present results it cannot be said which, if either, of these is the case as the EPSPs were not examined at depolarised levels of potential.

In keeping with other 'in vitro' studies action potentials could be elicited with sufficient levels of stimulation applied to the cortex. It was also observed that the input resistance during the time course of the EPSP was increased except for at the peak, and also that EPSPs were often succeeded by long-lasting depolarisations. These results confirm the earlier findings of Kita et al (1985a) who similarly found that the long-lasting depolarisations usually become more prominent with increased current intensity. They suggested that one of two mechanisms might be responsible for the long-lasting depolarisation. Firstly, a transient increase in extracellular  $K^+$  concentration created by the simultaneous excitation of many neuronal elements or secondly, it may be due to slow calcium-dependent potentials as the amplitude was decreased by a decrease in calcium in the medium.

The first mechanism seems somewhat unlikely in light of the evidence presented by Calabresi, Misgeld and Doherty (1987). The application of TEA appeared to have little effect on the long-lasting depolarisation although it was mentioned that plateau spikes could be elicited on top of the EPSPs if stimulation was sufficiently strong. A similar observation occurred in this study

after potassium currents were blocked from inside by using recording electrodes containing caesium chloride, such that long-lasting depolarising plateau potentials were elicited which lasted up to 140msec. Unfortunately, the involvement of a calcium conductance in the long-lasting depolarisation could not be confirmed by the present study as the effects of calcium-free medium on the cortical response were not examined. Therefore, further studies involving ionic manipulation are required to elucidate the exact mechanism underlying this potential.

An alternative explanation for the source of the long-lasting depolarisation came from Misgeld et al (1982). They observed this phenomenon only in the presence of barbiturates or 4-aminopyridine and suggested that it was a reversed IPSP. The fact that chloride-filled electrodes were used would be likely to reverse any chloride driven IPSP present. However, in the present study these potentials were present when either chloride- or acetate-filled electrodes were used, which tends to refute the claims of Misgeld et al (1982).

One further option also has to be considered. It has been found that glutamate, the putative transmitter of the corticostriatal pathway, induces a dose-dependent release of DA in striatal slices which is calcium-dependent (Roberts and Sharif, 1978; Roberts and Anderson, 1979). It also facilitates DA release in stimulated slices and this effect is abolished by glutamate diethyl ester (Rudolph et al., 1983). In addition, Chavez-Noriega et al (1986) found that frontal cortex stimulation induced a decrease in the terminal excitability of nigrostriatal dopaminergic neurons. It is possible therefore that cortical stimulation within the slice



releases DA from the nigrostriatal terminals severed from their neurons of origin. This DA could then act on the recorded neuron to elicit a response similar to that described by Wilson et al (1982) after destruction of all inputs to the striatum except that from SN.

The duration of the long-lasting depolarisation was increased by DA in this study which, if the above argument is correct, could be due to the applied DA replacing that depleted from the terminals by cortical stimulation, or contributing an additional effect. Alternatively it could have been due to the fact that higher currents were required to elicit a response after DA and as already mentioned, the duration of this long-lasting depolarisation is greater with larger current intensities. If Kita et al (1985a) are correct, and the long-lasting depolarisation is due to a slow calcium-dependent potential then it may be possible that DA increases the duration of this depolarisation via a facilitatory effect on the calcium current. This possibility requires further study perhaps by the use of voltage clamping to identify the nature of the ionic mechanism and the exact effects of DA on it.

As mentioned previously it had been hoped that the effects of DA on corticostriatal transmission would be distinguished pharmacologically. However, as already explained, this was not possible. 'In vivo' studies which have attempted this elucidation have implied that DA might have a presynaptic effect via D2 receptors (Mercuri et al., 1985; Brown and Arbuthnott, 1983). While the present study does not offer any new evidence on this matter, it does appear that DA is having an inhibitory effect by increasing the current threshold required to elicit a response. Whether this effect is via a pre- or post-synaptic mechanism remains to be elucidated.



### Significance of the DA effects in striatum

The AHP has been postulated to be involved in the prevention of prolonged neuronal discharge thereby contributing to the accommodation of action potential firing (Madison and Nicoll, 1984; Kernell, 1985; Calvin and Schwindt, 1972; Schwindt and Calvin, 1973; Baldissera and Gustafsson, 1970, 1974a, 1974b; Meech, 1978; Barnett et al., 1980; Partridge, 1981). Thus it appears that striatal neurons also possess this effective mechanism for the control of spike discharge, which can be affected by DA.

Bearing this in mind, the present results show that DA appears to have two major effects on striatal neuron firing.

1. Inhibition via an increase in the threshold for spike activation.
2. Excitation via a decrease in the AHP and subsequent decrease in spike firing accommodation.

These effects did not appear to be related as AHPs were only observed when action potentials were evoked and were dependent on the intensity of neuronal firing.

A similar situation has been observed after the application of serotonin on hippocampal neurons (Colino and Halliwell, 1987) where the two effects were found to be mediated via different receptor subtypes. Certainly Akaike et al (1987) have described inhibitory and excitatory effects of DA in the striatum which were mediated via D1 and D2 receptors respectively. However, the effects of DA on the AHP were not examined. It is thus interesting to speculate that the effects of DA may be mediated in a similar manner to those of serotonin, but further investigations would be required.

It has been suggested that endogenous DA may not act as a classical neurotransmitter but instead may exert a "neuromodulatory" effect on target neurons (Chiodo and Berger, 1986; Gribkoff and Ashe, 1984; Moises et al., 1979; Rolls et al., 1984; Schneider et al., 1984). The present results lend weight to this hypothesis, such that DA could exert both a facilitatory and an inhibitory effect on the firing of action potentials which could depend on the type of receptors activated and also the background stimulation received by the neurons. This could therefore help to explain the contradictory reports about its action which were outlined earlier (see Introduction).

#### SUMMARY

1. Properties of striatal neurons recorded intracellularly 'in vitro' are similar to those described in other 'in vitro' and 'in vivo' studies.
2. DA exerts an inhibitory effect on both intracellularly and cortically evoked action potentials in striatal neurons without changes in membrane potential.
3. The presence of a distinct long-lasting AHP has been demonstrated.
4. DA decreases the AHP amplitude together with a reduction in the accommodation of striatal neuron firing during intracellular current injection.

## Concluding Remarks

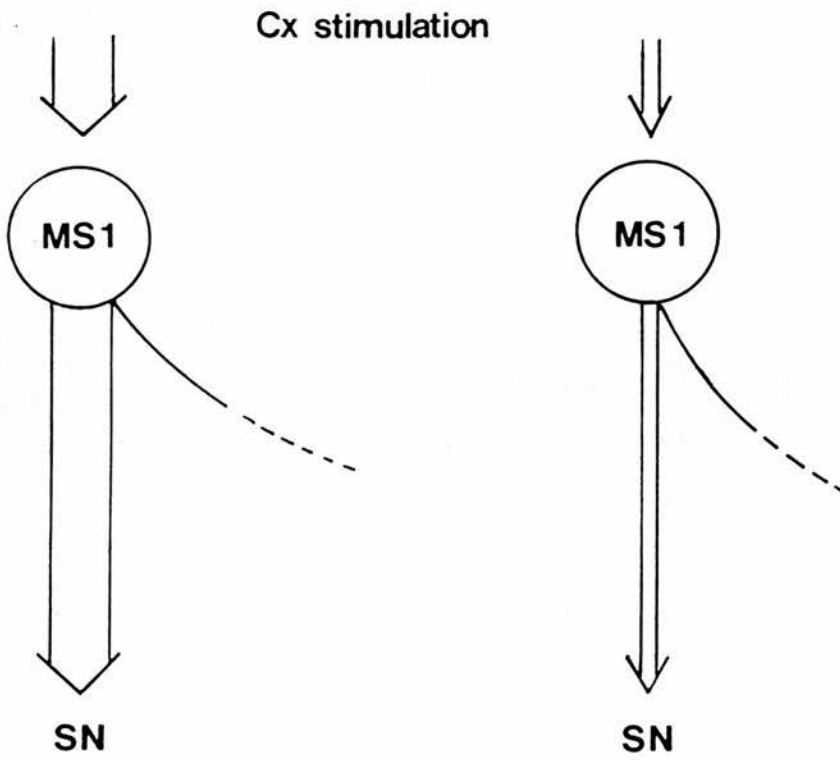
As demonstrated in Chapter 5, the effect of DA on single striatal neurons appears to be twofold, namely that it has an inhibitory effect on firing threshold and may also be excitatory by reducing the AHP amplitude and hence accommodation. This, therefore, is a possible means of explaining the contradictory reports about its action in previous studies (see Chapter 5, Introduction).

This finding can be examined in broader terms in relation to the cortical input to a typical striatal neuron (e.g. a medium spiny neuron). If this input is of short duration then the striatal neuron might be close to its firing threshold and so the effect of DA would be to increase this threshold and thus appear inhibitory, as was demonstrated in Chapter 5. If, on the other hand, a sustained cortical input occurred, a prolonged firing of the striatal neuron would take place and so DA would further facilitate the firing.

Consider a neighbouring pair of striatal neurons receiving "threshold" and "prolonged" cortical inputs, then their activities would be different, and hence the effect of DA on the two neurons might also be different (see Figure 65). As a result, the action of DA might be considered to give rise to an enhancement of the cortical signal such that DA itself may be thought of as a "contrast controller".

Of particular relevance to the present study was the finding by Rolls et al (1983) that in conscious monkeys, the prefrontal cortex and anterior striatum (the areas studied in this thesis) were

Before DA



After DA

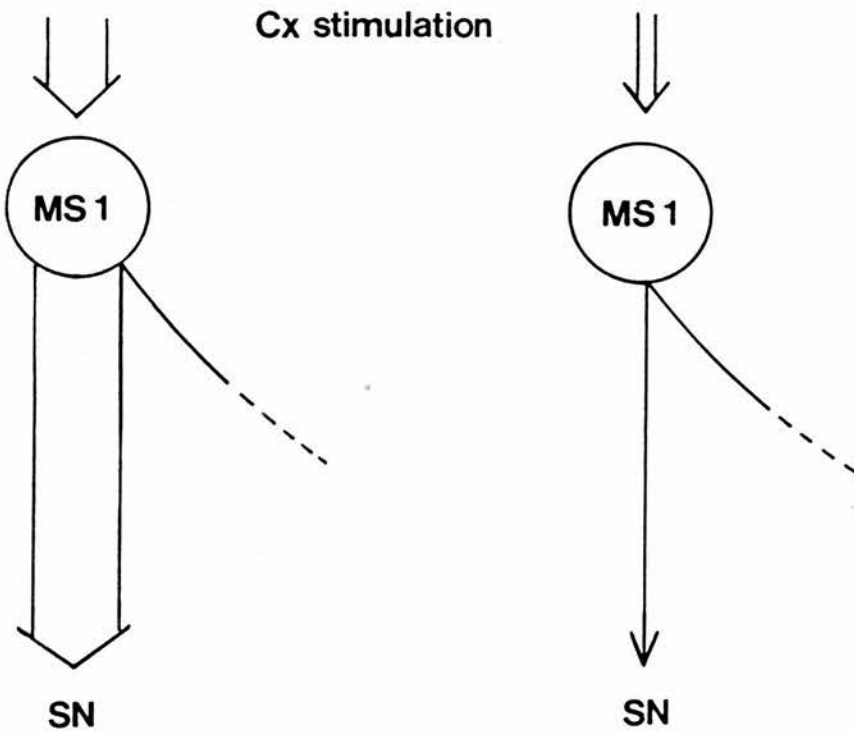


FIGURE 65. Proposed "contrast" mechanism by which DA modulates the activity of medium spiny striatal neurons. See text for explanation.

involved in the initiation of movement, although, more importantly, these neurons only responded to environmental stimuli when they were significant to the behaviour of the animal. In addition, this was confirmed to be the case for some putaminal neurons which only responded to the click of a solenoid when it indicated that a fruit juice reward could be obtained (Evarts and Wise 1984).

Interestingly, Schultz et al (1983) presented evidence to suggest that some SNc neurons behave in a similar manner and respond maximally at the initiation of a movement. Rolls' group also suggested that DA may increase the "signal to noise ratio" in striatal neurons as a result of iontophoretic experiments carried out in behaving monkeys (Rolls et al 1981). This action is very similar to that described above which derived from our 'in vitro' experiments.

Rolls et al (1983; 1987) suggested that the anterior striatum contains neurons which are important for the utilisation of environmental cues for the preparation for a behavioural response and for particular behavioural responses made in certain situations to certain environmental stimuli. They also postulated that different neurons in the cue-related group respond to different subsets of environmentally significant events and thus convey information useful in switching behaviours in preparing to make different responses, a role which might be helped by the proposed "contrasting" effect of DA.

It may also be possible that, besides controlling the contrast between the activity of individual neurons, DA may also play some effective part in controlling the contrast between groups of neurons such as those contained within the patch and matrix

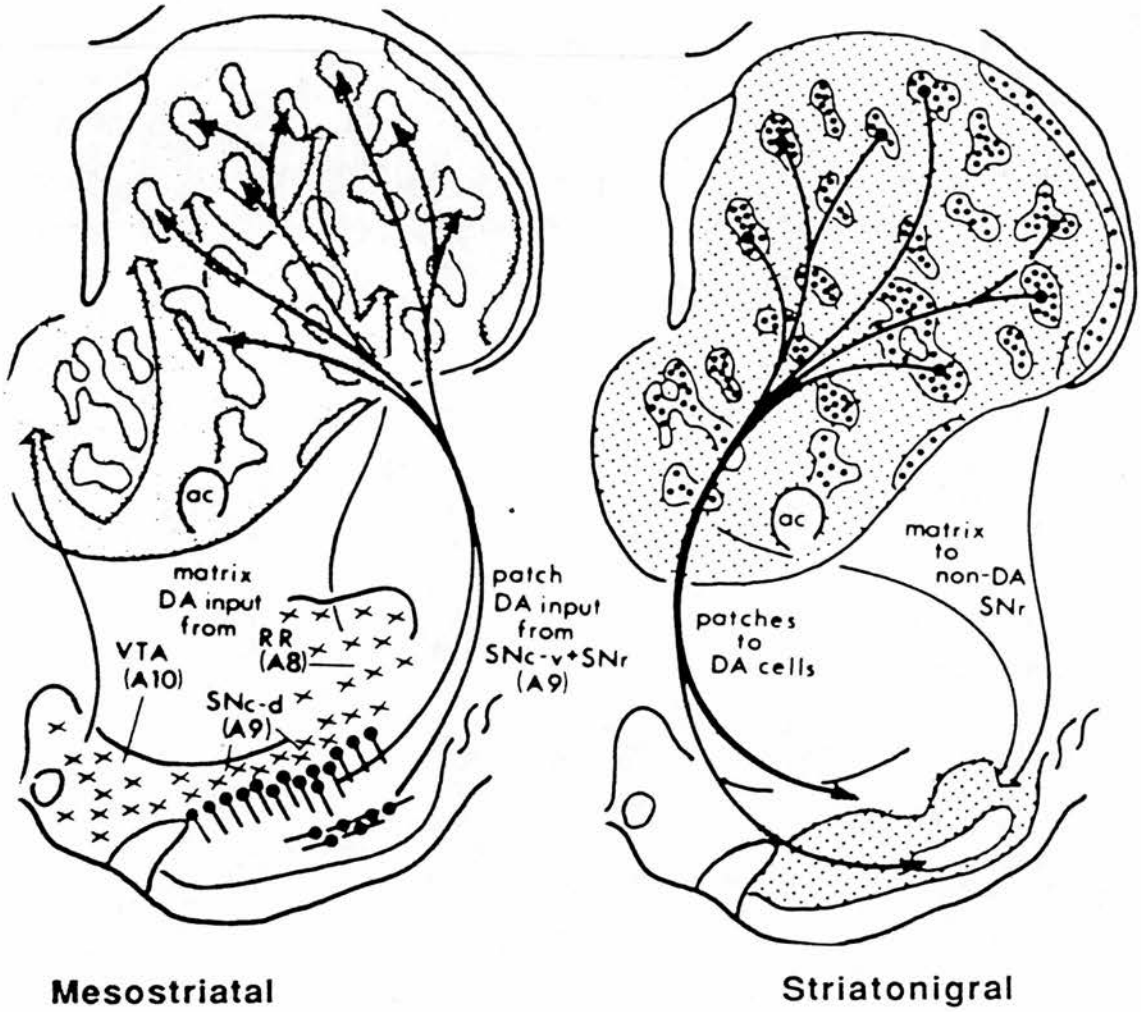


FIGURE 66. Comparison of the organisation of mesostriatal and striatonigral systems in the rat.  
Taken from Gerfen (1987)

compartments of the striatum. Furthermore dopaminergic inputs to the striatum are also heterogeneous (Gerfen, 1984; 1985; Gerfen et al, 1985) and respect the patch/matrix organisation.

In light of this proposition, the results presented in Chapter 2 indicate that some areas within prefrontal cortex (that is, prelimbic, anterior cingulate, etc) project to patches or matrix. Gerfen has also shown that patches in turn project to the location of DA perikarya and their proximal dendrites in the ventral tier of the SNc (SNc-v) and the SNr while the matrix projects to the SNr (non-DA neurons) and to SNc DA neurons only by way of their dendrites. Dopaminergic inputs to matrix originate from the ventral tegmental area (VTA), dorsal tier of the pars compacta (SNc-d) and the retrorubial area (RR), but dopaminergic inputs to patches appear to originate from SNc-v neurons and from the A9 DA neurons located in SNr (Gerfen, 1984; 1985; 1987; Gerfen et al, 1985; Jimenez-Castellanos and Graybiel, 1987) that is from the very cells receiving input from the patches. These inputs and outputs are summarised in Figure 66.

A diagram of adjoining patch and matrix areas is shown in Figure 67 to illustrate the proposed effects of DA on these systems. If input from prefrontal cortex to, for example, the patch area is sustained while that to matrix is only short or less intense then the patch neurons are likely to fire more rapidly than those in matrix so that DA neurons in SNc and SNr would be likely to be activated by this sustained input. As a result, DA would be released in striatum in mainly patch areas and would thus enhance the activity of the neurons therein by decreasing the AHP and accommodation. This would result in an increase in the contrast



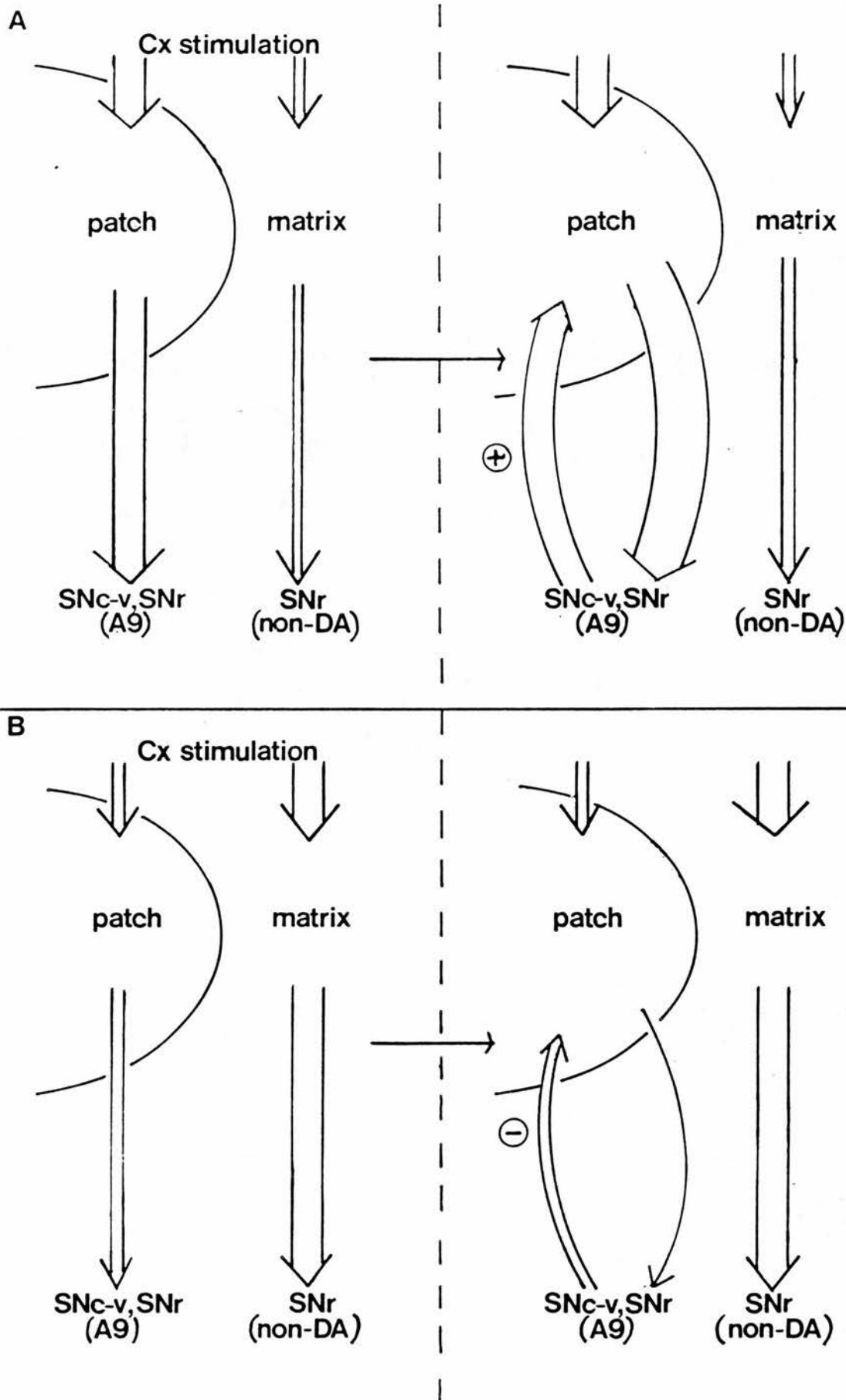


FIGURE 67. Possible mechanism by which DA increases the contrast between the activity of neurons in the patch and matrix compartments of the striatum. See text for explanation.

between the activity of neurons within the two compartments by a type of positive feedback mechanism operated solely in connection with the patches. If on the other hand the input to the patch was low then the neurons would only send a small signal to the DA neurons in SN with the result that the DA released would inhibit the patch neurons, thus eliciting a negative feedback.

This "internal computational" arrangement would therefore provide a means of contrasting neuronal activity by way of DA altering only the activity within patches, but would not act on matrix neurons as these are thought not to send a major projection to SN DA neurons. In other words, the control of DA output and hence activity contrast would not be amplified in the matrix compartment by striatal neurons. Local axon collaterals from medium spiny neurons, dendrites of these neurons (which have been shown to span compartmental boundaries (Bolam et al, 1988)), or interneurons may allow the activity of patches to indirectly affect the activity of matrix neurons by way of these interconnections. An alternative mechanism for the control of contrast in the matrix may come from direct inputs on to DA cells from, for example, prefrontal cortex or thalamus.

#### Disturbances of DA function

The striatum has outputs to motor systems via GP and SN. The DA input from SNc is important for the proper functioning of the striatum in that the degeneration of this pathway in man occurs in Parkinson's disease where there is amongst other deficits, akinesia (i.e. inability to initiate voluntary movements - Hornykiewicz, 1973), while its destruction in animals leads to catalepsy and an

inability to orient to environmental stimuli (Iversen, 1979; 1984; Marshall et al., 1984).

It is proposed therefore that DA is required to control the contrast between the activity of different individual neurons or groups of neurons in order to maintain normality of function. If DA is absent then no enhancement of the contrast between two sets of inputs occurs. Consequently, in the case of the initiation of movements or behaviours, no one specific message is relayed through the striatum to output structures so that a specific action in response to relevant environmental cells cannot be initiated.

This theory requires further investigation but provides an interesting prospect for future studies on the striatum and its role in behaviour.

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### The rat cortico-striatal pathway *in vitro*

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There is now good anatomical (Freund, Powell & Smith, 1984) and physiological evidence (Brown & Arbuthnott, 1983) that an important function of the nigro-striatal dopamine system is to control the action of the cortical input to the striatum. Quantitative pharmacological analysis and intracellular investigation of the effects of the dopamine input are very difficult *in vivo* and so we have developed an *in vitro* slice preparation of the cortico-striatal pathway.

The slices were prepared from the brains of young male albino rats (120–180 g), killed by decapitation. The brains were hemisected and parahorizontal blocks of tissue cut from one hemisphere at an angle of 15° to the cortical surface. Slices (approx. 300  $\mu$ m thick) were cut from these blocks and then transferred to the recording chamber in which they were maintained at 37 °C in a moist atmosphere and superfused at 0.5–1.0 ml/min with a solution containing NaCl 124 mM, KCl 3.3 mM, MgSO<sub>4</sub> 2.4 mM, CaCl<sub>2</sub> 2.5 mM, KH<sub>2</sub>PO<sub>4</sub> 1.25 mM, NaHCO<sub>3</sub> 13 mM, glucose 10 mM, saturated with 95% O<sub>2</sub> 5% CO<sub>2</sub>.

Stimuli were applied to a stainless steel wire placed on the surface of the slice on the fibres of the corpus callosum and recordings made from the grey matter of the striatum close to a bundle of cortico-fugal fibres. Extracellular and intracellular recordings were obtained with electrodes filled with Pontamine Sky Blue in 0.5 M sodium acetate (resistance 80–100 M $\Omega$ ) or 3 M-KCl (60–80 M $\Omega$ ). The fibre bundles were visible for several millimetres and it was possible to plot the recording position with respect to the stimulating electrode. Cells responding to cortical stimulation occurred in clusters which may be a physiological correlate of the anatomical distribution of the cortical input (Gerfen, 1984).

From 109 cortically driven cells in the slices, 10% showed the spontaneous activity typical of striatal cells *in vivo*. Of 212 striatal units similarly identified *in vivo* we previously found 16% to have such activity. The typical response to cortical stimulation in both situations is a short-latency excitation in the striatal cells which can be seen to result from a sharply rising depolarizing synaptic potential in intracellular records. In slices, where the conduction distance and time are both amenable to measurement, we estimate a rate of transfer of activity from cortical stimulation to striatal cell response of 0.11 m s<sup>-1</sup>.

Thus the properties of the cortico-striatal system have been preserved in these slices, in which it will be possible to investigate more fully the physiological role of dopamine in the striatum.

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## Electrophysiological demonstration of host cortical inputs to striatal grafts

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Adult rats received a unilateral injection of ibotenic acid into the striatum, resulting in an extensive loss of intrinsic striatal neurones. Transplanted striatal cells from embryonic day 14 rat foetuses grew well in the damaged tissue and approximately 6 months later brain slices were cut in such a way as to include the grafts and nearby cortical tissue. Electrophysiological recordings from the grafts showed typical extracellular responses to stimulation of the subcortical white matter. Thresholds and latencies for responses were similar in grafted and control striatum. The grafted cells, however, appeared to be subject to less collateral inhibitory control than normal striatal neurones. The similarities between the responses of grafted neurones and those in normal striatum suggest that corticostriatal connections reform after transplantation, which may be an important component in the behavioural recovery observed in similar animals.

In recent years much attention has been focused on the transplantation of foetal brain tissue as a means of repairing damaged neuronal circuits [6, 16, 22, 25] to study neuronal development and functional organization within the central nervous system. In particular, dopamine (DA)-rich grafts have been found to compensate for behavioural deficits induced by neurotoxic lesions of the nigrostriatal DA pathway in adult rats [5, 10–15, 27]. More recently, several studies also indicate that foetal neostriatal tissue can survive, grow and reverse behavioural deficits induced by ibotenic acid- or kainic acid-induced lesions in adult rat striata [8, 9, 18, 20].

Striatal grafts in the ibotenic acid-lesioned striatum are of particular interest, because this model system provides the clearest evidence of functional recovery dependent upon reciprocal incorporation of the grafts into the host neural circuitry [7, 14]. The internal organization and connections of these grafts have been well characterized anatomically and neurochemically [8, 18, 19, 28], as well as behaviourally [7, 9,

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20], but there have been no studies indicating whether their anatomical connections are functional at the electrophysiological level, an approach which has provided important information on the functional connections of DA grafts in the striatum [2, 31] and cholinergic or noradrenergic grafts in the hippocampus [4, 21, 30]. Whereas the majority of these electrophysiological recordings have been made *in vivo*, identification of the exact position of the graft and stability of the preparations can prove difficult. *In vitro*, slice preparations overcome both of these problems, and Segal et al. have used this approach successfully to identify the extent and pharmacological specificity of cholinergic fibre outgrowth from septal grafts in hippocampal slices [30].

In the present study we demonstrate functional inputs to striatal grafts from the neocortex *in vitro*, and compare the response of grafted striatal cells to activation of this input with the response observed with normal striatal cells in control animals.

The experiments employed 16 young adult female Sprague-Dawley rats. All ani-

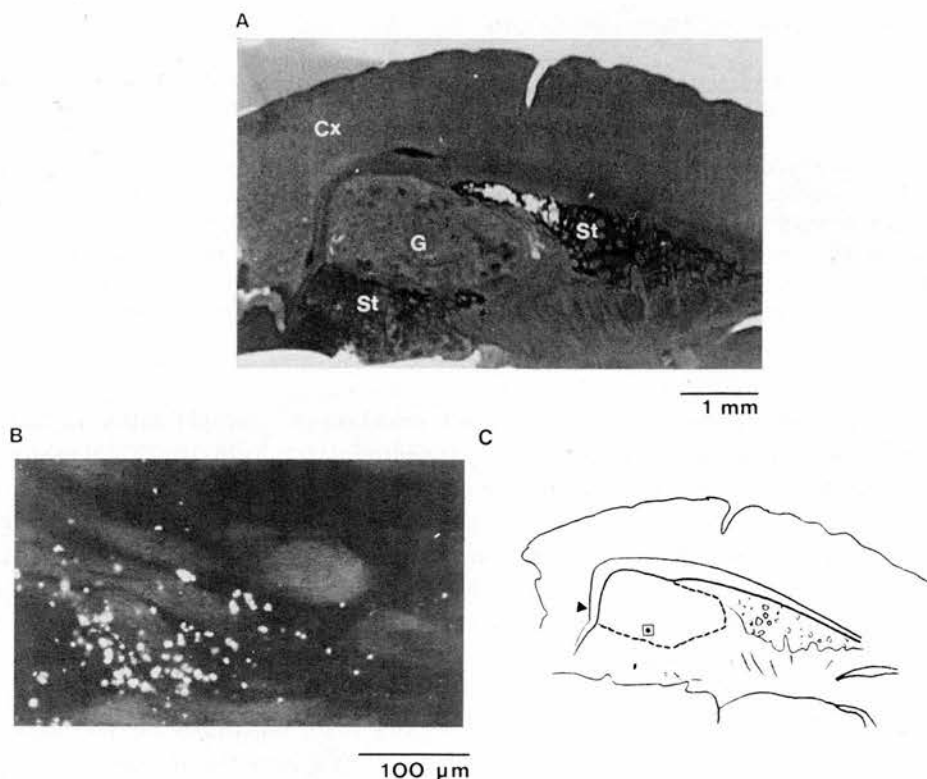


Fig. 1. Striatal grafts. A: photograph of a 50  $\mu$ m section taken from a tissue slice after the recording experiment, containing cortex (Cx) and striatum (St). The graft (G) is also clearly visible. The section has been stained using standard acetylcholinesterase procedures. B: high-power photograph of a section taken from a slice similar to that shown in A. Neurons fluorescently labelled with rhodamine-latex microspheres are visible within the graft. C: diagram representing relative positions of recording (star) and stimulating (arrowhead) sites within the slice. The boundary of the graft is represented by the dotted line.

mals initially received unilateral striatal lesions under Equithesin anaesthesia, by stereotaxic injection of  $1.0\ \mu\text{l}$  of  $0.05\ \text{M}$  ibotenic acid into the neostriatum, at two sites [13]. Approximately 2 weeks later, the striatal eminence was dissected from 13 mm embryos of the same rat strain, and prepared as a dissociated cell suspension. Deposits of  $3\ \mu\text{l}$  were implanted into the striatum of all adult lesioned hosts, half way between the two lesion injection sites [13]. In half of the host animals, the embryonic graft tissue was labelled with rhodamine-fluorescent latex microspheres, at a concentration of one embryonic tissue piece per  $5\ \mu\text{l}$ , whereas no similar label was applied to the grafts in the remaining hosts [1]. An additional 4 young adult female rats were used as controls for recording from the intact striatum. The viability of all grafts was confirmed at the end of the recording experiments by standard Nissl and acetylcholinesterase histochemistry (see Fig. 1A).

Six months after transplantation rats were killed by decapitation and the brains rapidly removed and rinsed in ice-cold medium (composition in mM: NaCl 124, KCl 3.3,  $\text{MgSO}_4$  2.4,  $\text{CaCl}_2$  2.5,  $\text{KH}_2\text{PO}_4$  1.25,  $\text{NaHCO}_3$  26, glucose 13). They were then hemisected and parahorizontal blocks of tissue were cut from the graft-containing hemisphere at an angle of  $15^\circ$  to the horizontal, in order to maintain corticostriatal connections [3]. Using a Vibroslice, approximately  $300\ \mu\text{m}$  thick slices were cut from these blocks while the tissue was continually moistened with oxygenated medium (95%  $\text{O}_2$ –5%  $\text{CO}_2$ ) at room temperature. The slices were then transferred to the recording chamber where they were placed on a nylon mesh and left for one hour before recording. This whole procedure took no more than 5–7 min.

The bathing medium flowed into the chamber under gravity and was removed by aspiration. A flow rate of approximately 1 ml/min was maintained. The medium was continually bubbled with a 95%  $\text{O}_2$ –5%  $\text{CO}_2$  gas mixture and an oxygen-rich atmosphere was maintained around the slice in the recording chamber. The temperature of the bath was maintained thermostatically at  $36^\circ\text{C}$ . Extracellular recordings were performed in tissue slices from the grafted striatum as well as from control neostriatal slices (see Fig. 1B). In many, but not all, cases the electrode was filled with 2% Pontamine sky blue, (in  $0.5\ \text{M}$  sodium acetate, 50–80  $\text{M}\Omega$ ) and the slices viewed under ultraviolet illumination for visualization of the rhodamine-labelled grafts (see Fig. 1C), in order to accurately determine placement of the electrodes within the graft tissue. Recording glass micropipettes were also occasionally filled with  $3\ \text{M}$  KCl (30–50  $\text{M}\Omega$ ).

Stimuli (0.1–100 V, 0.1 ms pulse width, 1 Hz) were applied to a stainless-steel wire placed on the surface of the slice at the edge of the corpus callosum nearest the cortex (see Fig. 1C). In all cases the recording electrode was at least 1.0 mm from the stimulation site. Control experiments involving intracellular recording from striatal neurones demonstrated that direct stimulation by current spread could only be detected with stimulus intensities greater than twice as high as any employed in the present series (A. Rutherford, unpublished observations). Additionally direct antidromic stimulation results in response latencies less than 1 ms, whereas all latencies observed here were more than 2 ms.

Cell activity was amplified and displayed on an oscilloscope. Responses to paired

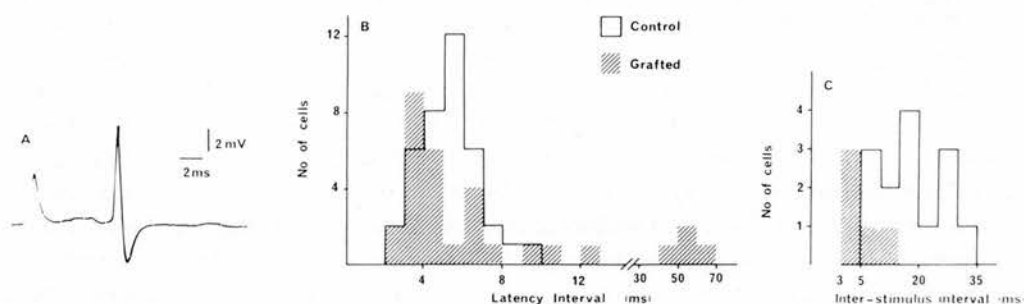


Fig. 2. Responses to cortical stimulation. A: typical response of a grafted cell. B: histogram showing a comparison between response latencies of grafted neurons and normal striatal neurons. C: histogram of the shortest inter-stimulus intervals necessary to still elicit action potentials after cortical stimulation. Shading indicates the responses of grafted cells.

stimuli delivered at various intervals (1–250 ms) were also examined.

Successful extracellular recordings were obtained from the brain slices of 8 grafted rats (30 neurones) and all 4 controls (38 neurones). None of the neurones in either group showed any spontaneous activity. Rather, they were all identified by their response to cortical stimulation (see Fig. 2A), and, although the density of cells in the grafts is relatively normal [19], the number of grafted cells identified in this manner compared to those in control striatum was very much lower. This therefore suggests that a smaller proportion of grafted neurones are responsive to cortical activation.

The firing characteristics of the grafted and normal striatal neurones in vitro is shown in Table I. Twenty-six of the 30 grafted cells were directly comparable with control striatal neurones in the threshold and latency of the response. The remaining 4 graft cells showed quite distinctive abnormally long latencies (see Fig. 2B). We cannot tell from the present data whether these abnormal late responses are attributable to polysynaptic connections, disinhibition of a normal slow cortical input, or the presence of an abnormal slow cortical input.

TABLE I

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF GRAFTED AND NORMAL STRIATAL NEURONS

Thresholds and latencies for responses produced by cortical stimulation are similar in both groups. The minimum inter-stimulus interval necessary to still produce two action potentials after cortical stimulation was significantly different,  $*P < 0.001$ .

	Control	Grafted
Threshold (mA)	$0.51 \pm 0.22$ ( $n = 38$ )	$0.41 \pm 0.27$ ( $n = 30$ )
Latency (ms)	$5.03 \pm 1.48$ ( $n = 38$ )	$4.78 \pm 2.45$ ( $n = 26$ ) $51.00 \pm 9.87$ ( $n = 4$ )*
Inter-stimulus interval (ms)	$17.71 \pm 8.52$ ( $n = 14$ )	$5.6 \pm 3.21$ * ( $n = 5$ )



Normal striatal neurones show an initial excitation followed by prolonged inhibition after cortical stimulation [17, 23]. In order to test the inhibitory phase of the cortical response we varied the intervals between pairs of cortical stimuli over 1–250 ms, using a stimulus intensity 1.5 times greater than that required to produce the initial response. As shown in Fig. 2C, the grafted neurones had significantly shorter inter-stimulus threshold intervals than the control cells even in our small sample (Mann–Whitney  $U = 5$ ;  $P < 0.001$ ; see Table I). All 5 neurones tested in the grafts had latencies to cortical stimulation (mean 3–4 ms, range 2–4.4) similar to those found in control slices.

These observations provide clear evidence that a subpopulation of neurones in striatal grafts receive a cortical input that is relatively normal in terms of the parameters of the primary response. The abnormal responses to short paired-pulse intervals suggest, however, that the grafted cells may lack important intrinsic inhibitory regulation. The 'post-activation inhibition' of striatal cells is a distinctive characteristic of these neurones *in vivo* [17, 23]. The inhibition has normally been thought to be a result of intrastriatal mechanisms, and Park et al. [26] have evidence that it may be a property of recurrent collateral activation providing a self-inhibition. Our results suggest that either this mechanism does not develop within the grafts, or that our method of stimulation does not activate the local inhibitory mechanisms in the transplants.

It is not simply the case that this paired-pulse phenomenon can be attributed to a lack of GABAergic neurones within the grafts, since both postmortem [19] and *in vivo* biochemical studies [29] have recorded extensive GABA activity in the grafts and their efferent connections. Alternatively, it has been suggested that striatal grafts become arrested at a relatively early postnatal stage of development [14], whereas in the cat the prominent IPSP which follows cortical stimulation develops relatively late in the postnatal period [24]. Another possibility arises from the reduced population of grafted striatal neurones which respond to cortical stimulation, with the consequence that collateral inhibitory influences would be very much reduced.

The present electrophysiological results provide a powerful supplement to previous anatomical studies of the connectivity of striatal grafts [14, 28]. Thus, although many of the normal afferent connections of the striatum (e.g. from substantia nigra and thalamus) have been shown to reconnect with striatal grafts, a corticostriatal input has proved harder to demonstrate. Our sample of recorded cells from grafts were predominantly found within 200  $\mu\text{m}$  of the graft–host border, whereas the anatomical studies exclude cases where the tracer invades an area so close to the graft border in order to exclude problems of extra-graft leakage [28]. This then suggests that although striatal grafts receive a cortical input from the host brain, this afferent system undergoes less extensive collateral regeneration than is seen, for example, in ascending monoamine systems [28].

Striatal grafts similar to those employed in the present experiments have been found to ameliorate lesion-induced deficits in complex cortically dependent tasks such as delayed alternation learning, as well as in tasks that may simply reflect activation of striatal outputs [20]. This has been taken to suggest the possibility that striatal

graft tissue may actually serve to reconstruct damaged cortico-striato-pallidal connections [7]. The present results provide direct evidence that functional connections do become established between the host neocortex and grafted striatal cells in the lesioned rat striatum.

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## An afterhyperpolarization recorded in striatal cells 'in vitro': effect of dopamine administration

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**Summary.** Parahorizontal slices of the neostriatum from young adult male rats were maintained at the fluid interface of a perfusion chamber. Intracellular recordings were made with potassium acetate filled micropipettes. The mean resting membrane potential of the neurones was  $-84$  mV and all produced action potentials which overshoot  $0$  mV. All the cells were silent at resting membrane potentials and only fired in response to depolarizing pulses delivered through the intracellular electrode. When long pulses were applied, the firing rate within the train of action potentials evoked was slower at the end of the pulse. In a very few cells at resting membrane potential, but in all cells depolarised from a holding potential of  $-65$  mV, the resulting train of action potentials was followed by a slow hyperpolarizing potential. The potential observed was smaller in amplitude than the afterhyperpolarization seen in hippocampal pyramidal cells in vitro, but its voltage sensitivity was similar. Iontophoresis of dopamine (DA) close to the recording pipette resulted in two distinct actions. Firstly, DA reduced the number of action potentials following a short pulse, by increasing the threshold for spike initiation. Secondly, it also inhibited the hyperpolarizing potential which followed trains of action potentials even when allowance was made for the increased firing threshold. The inhibition of this hyperpolarization was accompanied by an increase in the number of spikes per pulse. Whether these two actions of DA are mediated by a single receptor/membrane action is unclear. Nevertheless, they may help to reconcile the contradictory literature regarding the action of DA upon extracellular responses in striatal cells. When threshold effects are studied the action of DA will be inhibitory. On the contrary an

excitation will be seen when trains of action potentials follow activation of the cells, since the reduction in the afterhyperpolarization by dopamine will result in decreased accommodation and more spikes per depolarizing stimulus.

**Key words:** Striatum – Dopamine – Afterhyperpolarization – Basal ganglia

### Introduction

The electrophysiological properties of hippocampal cells have been well characterised in studies of brain slices maintained in vitro (Kandel and Spencer 1961; Schwartzkroin 1975; Langmoen and Andersen 1981). Although the hippocampus is not considered to be a major part of any dopaminergic system, there is some evidence of a mesencephalic dopamine (DA) containing projection (Swanson 1982) and so hippocampal slices have been treated as a model system in which to examine the postsynaptic actions of DA. Bernardo and Prince (1982) found that dopamine augmented the afterhyperpolarization (AHP) which follows spike trains in these neurones. They suggested that effects of DA were mediated by an increase in intracellular calcium concentration and an activation of a calcium-dependent potassium conductance. On the other hand, Stanzione et al. (1984) reported that DA attenuated calcium-mediated events such as bursting activity and AHP. Since then, other equally contradictory studies have followed (Pockett 1985; Malenka and Nicoll 1986). From these studies therefore it seems that the major effect of DA in the hippocampus is on the calcium-activated potassium conductance underlying the slow AHP, although the direction of the change is debated.

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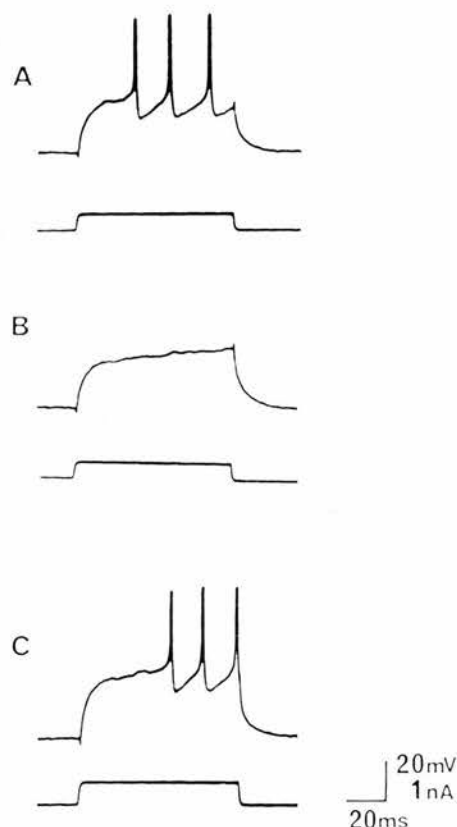
In the striatum, where the majority of DA neurones terminate, some details of the electrophysiology have been elucidated using the *in vitro* slice preparation (Kita et al. 1985a, b; Cordingley and Weight 1986; Cherubini and Lanfume 1987; Calabresi et al. 1987b). For instance, it has been shown that the fast action potentials recorded from striatal neurones can be abolished by tetrodotoxin (TTX). Once the sodium channels are blocked, voltage-dependent calcium conductances that underlie calcium potentials are revealed (Kita et al. 1985b; Calabresi et al. 1987b; Cherubini and Lanfume 1987). Furthermore, in the presence of tetraethylammonium, striatal cells show a separation of calcium and sodium-dependent regenerative potentials, and low threshold calcium spikes similar to the ones described for the thalamus and substantia nigra are observed (Calabresi et al. 1987b). So far, it has been reported that DA application reduces the frequency of action potentials evoked by a depolarizing pulse (Mercuri et al. 1985; Calabresi et al. 1987a), an effect which seems to be due to the activation of D1-DA receptors (Calabresi et al. 1987a). The application of a long duration current pulse has been reported to produce an AHP and accommodation in striatal neurones (Kita et al. 1985a) but the actions of DA on this response have not been studied.

We report here that the iontophoretic application of DA in striatal slices, apart from the known inhibitory effect on cell firing, also reduces the AHP following long depolarizing pulses. The action of DA on accommodation may reflect either or both of these effects.

## Material and methods

Male albino rats (120–150 g) were stunned and decapitated. The brain was rapidly removed and submerged in ice cold medium saturated with a mixture of 95% O<sub>2</sub> : 5% CO<sub>2</sub>. The medium was composed of (mM): NaCl 124, KCl 3.3, NaHCO<sub>3</sub> 2.6, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 2.4, KH<sub>2</sub>PO<sub>4</sub> 1.25 and D-glucose 10. The brains were hemisected on a platform covered by moistened filter paper and parahorizontal blocks of tissue (5 mm thick) were cut from one hemisphere at an angle of 15 deg to the cortical surface (Arbuthnott et al. 1985). The block of tissue was then affixed with cyanoacrylate to a teflon cutting platform and support was given by fixing a small agar block adjacent to one side of the tissue block. Once secured, the tissue was immersed in medium maintained at room temperature and further sectioned with a Vibroslice (Campden Instruments). The slices (300 µm) were then placed in the recording chamber on a nylon mesh covered with lens tissue. Warm medium (36°C) bubbled with the O<sub>2</sub>-CO<sub>2</sub> gas mixture flowed into the chamber under gravity, continuously superfusing the slice. The outflow of the chamber was removed by aspiration in order to maintain the slice on the interface between the medium and the warm moist gas mixture. The tissue was maintained in these conditions for 1 h prior to recording.

Glass microelectrodes filled with 2 M potassium acetate with a DC resistance of 60–90 MΩ were used. The signals were fed



**Fig. 1A–C.** Effects of iontophoresed DA on action potentials evoked by a depolarizing pulse applied to a striatal neurone. **A** Control, **B** 10 min after the onset of the application of DA, **C** the same number of spikes as in the control situation can still be evoked after DA if the stimulus intensity is increased

through a single microelectrode current-voltage clamp amplifier (Axoclamp 2) and displayed on an oscilloscope. The sample-and-hold amplifier was continuously monitored on a second oscilloscope. The capacity compensation was adjusted to give optimally rapid settling of electrode voltage during current injection. The switching frequency was adjusted while ensuring that the voltage decayed to baseline by the end of each cycle. Usually the sampling frequency was 3 kHz, 30% duty cycle. Bridge balance was checked repeatedly before and after cell impalement.

Recordings were photographed from the oscilloscope display or stored on FM magnetic tape for further analysis.

Dopamine (0.5 M, Sigma) was applied by iontophoresis (25–50 nA/3–5 min) from a double barrelled pipette placed as close as possible to the recording pipette. Sodium chloride (1 M) was used in the balance barrel. A retaining current of 10 nA was used between applications. TTX (1 µM, Sigma) was applied to the superfusing medium. In one experiment, calcium was substituted with magnesium (2 mM) in order to confirm the effect of calcium-free medium on the responses.

## Results

All intracellular impalements ( $n = 62$ ) were maintained in stable conditions for over 30 min with



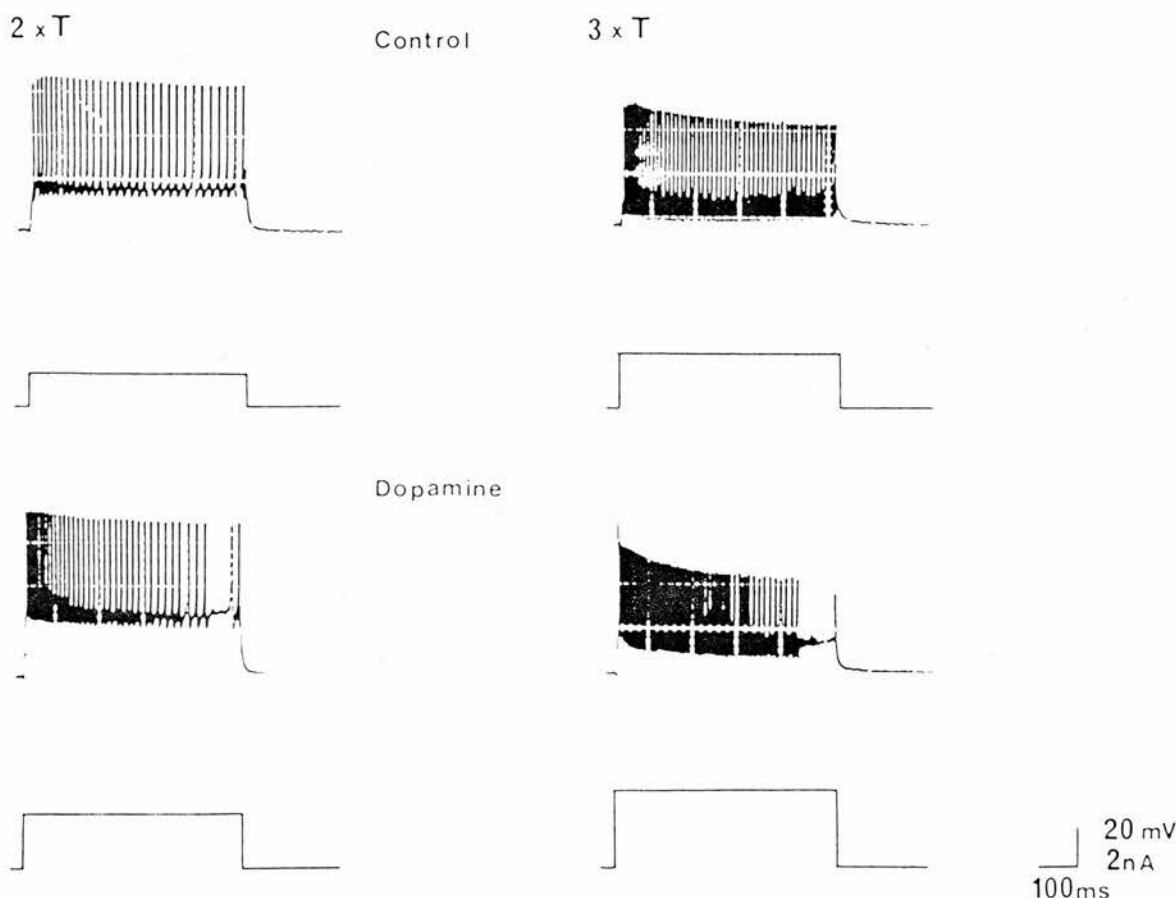


Fig. 2. DA increases the number of spikes induced by a depolarizing pulse at  $2\times$  and  $3\times$  threshold (T)

resting membrane potentials of at least  $-70$  mV (mean  $\pm$  standard deviation,  $83.7 \pm 6.4$  mV). No spontaneous action potentials were observed. When action potentials were elicited by rectangular depolarizing pulses of current ( $0.5$ – $1$  nA/ $80$  ms) they were greater than  $70$  mV in amplitude ( $81.7 \pm 7.1$  mV). When DA was iontophoresed, spikes evoked by depolarizing pulses were reduced in number or blocked. An increase in the amount of current injected, however, restored spike firing, indicating that DA acted on spike threshold (Fig. 1).

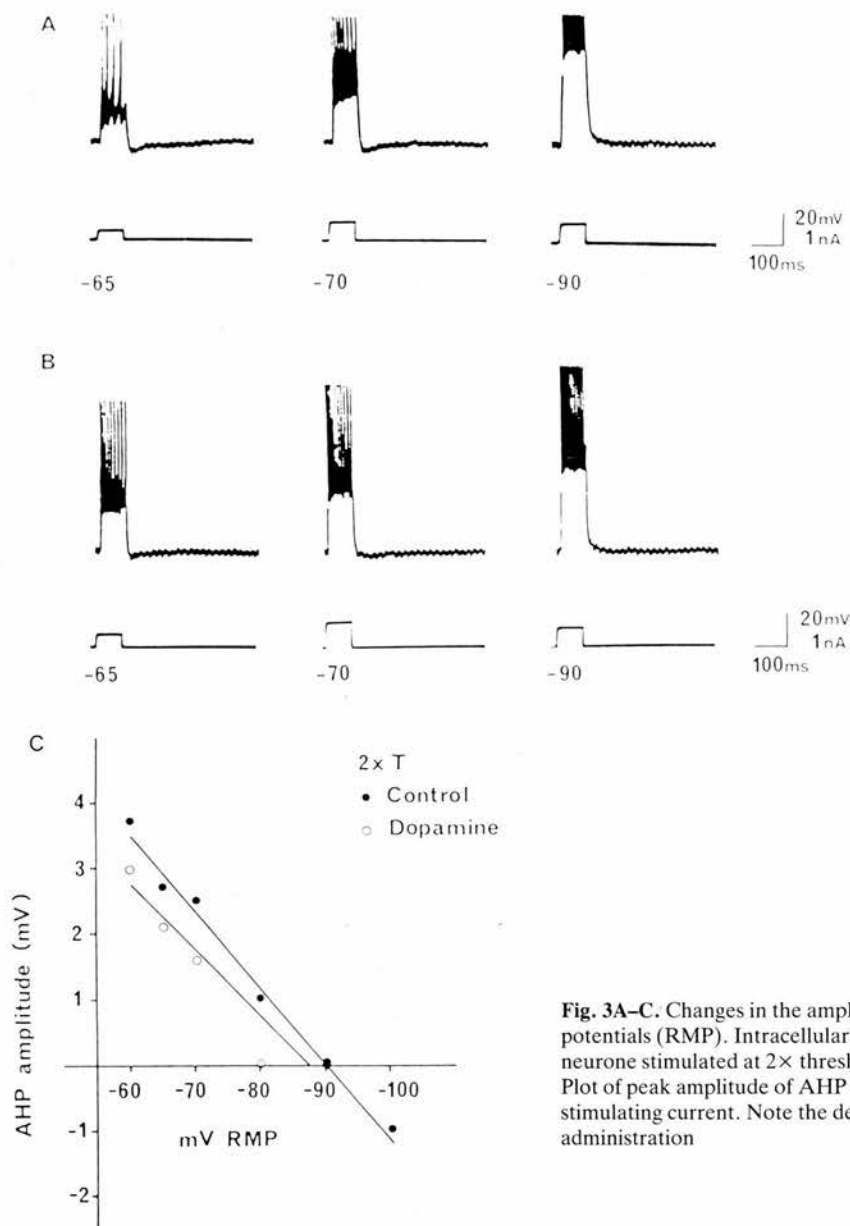
Throughout the experiment the intensity of the depolarizing pulse used was referred to the threshold current necessary to produce a single spike. The threshold was determined for each condition, i.e. before and after dopamine or at every holding potential.

Spike frequency accommodation was observed when long ( $500$  ms) current pulses were applied. Optimum accommodation was seen at  $2\times$  threshold; responses at  $1.5\times$  and  $3\times$  threshold were also studied. The rate of discharge was highest at the

beginning of the current application and gradually decreased with an accompanying decrease in spike amplitude and sometimes failure of spike generation towards the end of the pulse. With higher current intensity ( $3\times$  threshold), these phenomena became more accentuated and spike failure occurred more quickly in all  $8$  cells where it was tested (Fig. 2). For  $500$  ms depolarizing pulses of  $2\times$  threshold the mean number of spikes was  $30.3 \pm 9$  ( $n = 16$ ). After DA application equivalent depolarizations resulted in significantly more impulses ( $38 \pm 11$ ;  $p < 0.01$  paired 't') (Fig. 2).

Under resting conditions a brief depolarizing pulse ( $80$  ms,  $2\times$  threshold) evoked a train of action potentials with little AHP visible. However, as the cells were depolarized by steady d.c. current injection to potentials between  $-70$  and  $-55$  mV, further depolarization now evoked a single action potential or a train of spikes followed by a distinct AHP (Fig. 3).

The AHP was usually absent at levels between  $-80$  and  $-90$  mV, i.e. at resting membrane potentials.



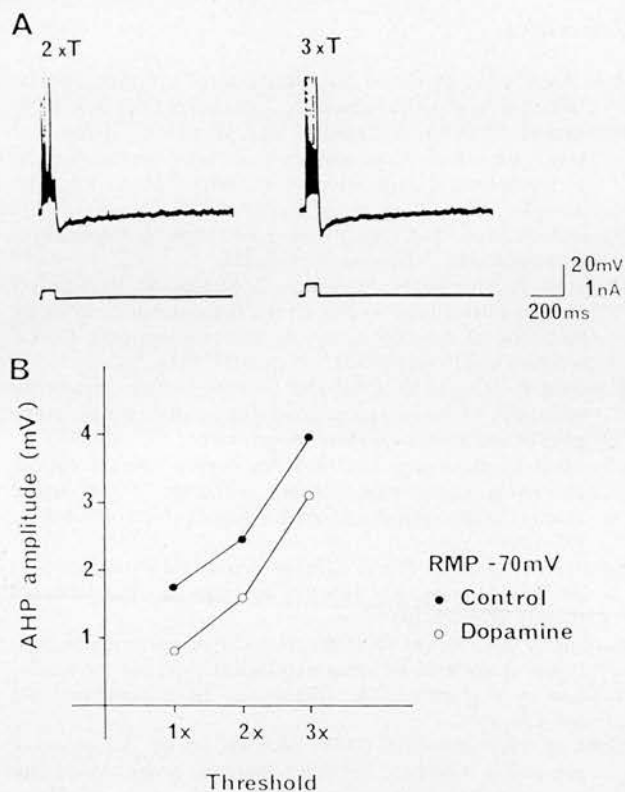
**Fig. 3A–C.** Changes in the amplitude of the AHP seen at different holding potentials (RMP). Intracellular current-clamp records obtained from a striatal neurone stimulated at  $2 \times$  threshold before **A** and after DA administration **B**. **C** Plot of peak amplitude of AHP voltage against membrane potential at threshold stimulating current. Note the decrease in amplitude of the AHP after DA administration

By plotting the peak amplitude of the AHP as a function of the holding potential (Fig. 3) a reversal potential of  $-90$  mV was obtained.

At a fixed membrane potential of  $-70$  mV the AHP ranged from 1 to 5 mV in amplitude and 200–1000 ms in duration, depending on the stimulus parameters. As the duration of a threshold depolarizing current pulse ( $0.42 \pm 0.2$  nA,  $n = 12$ ) was increased from 80 to 800 ms, AHPs of progressively larger amplitude and longer duration were evoked. When a depolarizing current pulse of a fixed duration (80 ms) and a variable intensity was used, it was also found that the AHP increased in amplitude with increasing depolarisation (Fig. 4). This was also

demonstrated by fixing the membrane potential to  $-70$  mV and changing the intensity of stimulation (Fig. 4B). DA reduced the size of the AHP as seen from the shift in the graph of AHP amplitude at different membrane potentials (Fig. 3C) and with various current intensities (Fig. 4B). On the 45 occasions when an AHP was elicited by equivalent stimuli at identical membrane potentials in the same cell before and after DA application, the reduction was highly significant ( $p < 0.001$ ; paired 't').

In two experiments the AHP was abolished after bath application of a medium in which calcium was replaced by an equimolar concentration of magnesium (2 mM) or to which TTX (1  $\mu$ M) was added.



**Fig. 4A, B.** Changes in the amplitude of the AHP at a fixed resting potential (RMP) of  $-70$  mV seen at different intensities of stimulation with a pulse of fixed duration (80 ms). **A** Representative intracellular current-clamp records at  $2\times$  and  $3\times$  threshold (T). **B** Plot of peak amplitude of AHP voltage against different intensities of stimulation

## Discussion

In confirmation of previous observations in cortical (Bernardi et al. 1987), striatal (Herrling and Hull 1980; Trulsson and Arasteh 1986; Calabresi et al. 1987a) and hippocampal neurons (Gribkoff and Ashe 1984; Stanzione et al. 1984; Malenka and Nicoll 1986), we have found that DA depresses the firing of striatal neurons in response to brief depolarizations without changing the membrane potential.

The main finding of our study is that DA reduces the AHP which follows repetitive firing in neostriatal neurones in a way perhaps similar to its action in the hippocampus (Stanzione et al. 1984; Malenka and Nicoll 1986). The AHP has been characterized for other neurones in the central nervous system. It is attributed to the activation of a calcium-dependent potassium conductance and is blocked by removal of external calcium (Wong and Prince 1981; Madison and Nicoll 1984; Malenka and Nicoll 1986; Constanti and Sim 1987; Lancaster and Nicoll 1987). It prevents prolonged neuronal discharge, thereby contributing to accommodation of action potential firing.

The amplitude and duration of the AHP observed under current clamp in striatal cells are comparable to those observed in the hippocampal (Gustafsson and Wigstrom 1981; Stanzione et al. 1984) or cortical neurones (Constanti and Sim 1987), even although temperature conditions were not optimal (Thompson et al. 1985; Lancaster and Adams 1986; Constanti and Sim 1987). In addition, the AHP of striatal cells, like that seen in other neurones was potential-dependent: it increased in amplitude with depolarization and decreased with hyperpolarization of the membrane (Hotson and Prince 1980; Gustafsson and Wigstrom 1981; Constanti and Sim 1987). As noted previously (Bernardo and Prince 1982), the reversal of the AHP was difficult to obtain, but, by plotting the amplitude of the AHP versus the holding potential, the reversal potential estimated was similar to that of neurones from other areas (Bernardo and Prince 1982; Constanti and Sim 1987).

The AHP has been reported to persist in the presence of TTX but only when currents large enough to evoke TTX-resistant action potentials were used (Hotson and Prince 1980; Gustafsson and Wigstrom 1981). TTX-resistant action potentials have been reported in striatal neurones and are presumed to be mediated by calcium (Kita et al. 1985b; Calabresi et al. 1987b; Cherubini and Lanfumey 1987). The block of the AHP by TTX in our experiment was seen only with current pulses insufficient to elicit calcium spikes.

It has been suggested that DA reduces the number of spikes induced by short depolarizing pulses by raising the threshold for activation of the voltage-dependent sodium channels sensitive to TTX (Stanzione et al. 1984; Calabresi et al. 1987a). We have seen that another effect of DA is to reduce the amplitude of the AHP. DA (Stanzione et al. 1984; Malenka and Nicoll 1986), serotonin (Madison and Nicoll 1982) and noradrenaline (Wong and Prince 1981; Lancaster and Adams 1986; Constanti and Sim 1987) show a comparable inhibitory effect on the hippocampal AHP. Noradrenaline acting on beta-adrenergic receptors eliminates the AHP and modifies spike frequency accommodation. This action is mimicked by 8-bromo-cyclic AMP which is an analogue of cyclic AMP; the effect is therefore thought to be mediated via a second messenger system (Lancaster and Nicoll 1987; Constanti and Sim 1987). The inhibitory effect of DA on striatal cells has been attributed to the action of D1 dopaminergic receptors and the subsequent stimulation of cyclic AMP (Calabresi et al. 1987a). It is possible, therefore that the action of DA on the AHP may also be mediated by D1 receptors, although we have no direct evidence to support this suggestion. Muscarine also



produces a reduction of the AHP which seems to be independent of cyclic AMP levels (Lancaster and Nicoll 1987; Constanti and Sim 1987). It is possible that DA-sensitive neurones in striatum also have muscarinic receptors (Nastuk and Graybiel 1985), but the action of acetylcholine on the AHP of these cells remains to be explored.

By acting on the voltage-dependent sodium channels and on the afterhyperpolarisation, DA could exert both a facilitatory and an inhibitory role on the firing of action potentials. This could explain some previous contradictory reports about its action. For instance, it has been shown that amphetamine and DA produce an increase in activity of striatal neurones recorded in vitro (Trulson and Arasteh 1986) or in freely moving animals (Trulson and Jacobs 1979), whereas in the anaesthetized preparation a predominantly inhibitory effect is observed (Groves et al. 1974; Siggins 1978). This difference in DA action could be due to the background stimulation received by the cells and/or to differences in their excitability. On one hand the increased threshold of the voltage-dependent sodium channels can result in an inhibitory effect. On the other hand, at high levels of stimulation, repetitive firing with little or no accommodation can be induced, which would be recorded as an overall excitation. This suggestion is supported by the fact that in low calcium/high magnesium medium striatal neurones in vitro exhibit rhythmic, irregular and bursting patterns of activity (Trulson and Arasteh 1986), perhaps the result of a decreased action of a calcium-activated potassium current underlying the AHP. During normal activities, when the membrane potential of neurones under dopaminergic influence reach firing threshold (as in the case of the chemically or electrically evoked depolarization) a burst of activity is elicited; for example bursting is usually associated with movement on the part of the animal (Trulson and Jacobs 1979). Thus, in conscious animals, DA release, by reducing the AHP might lead to an excitation resulting in an increased number of action potentials in each movement associated burst. In high calcium medium and in anaesthetised animals the cells are silent and the increased threshold for activation as a result of DA application will now seem like an inhibition of cell activity.

In conclusion we have demonstrated the presence of an afterhyperpolarization in striatal cells, which is decreased in amplitude by dopamine. This may result in a decrease in accommodation as revealed by an increased number of spikes per pulse. The present findings may help to explain previous contradictory reports about the electrophysiological effects of DA.

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***In vitro* study of the effect of dopamine administration on an after-hyperpolarization recorded in rat striatal cells**

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Dopamine (DA) has been shown to change several different membrane properties of neurones in, for example, hippocampus, substantia nigra and retina. We have tried to study its action on the cells of the neostriatum, which receive a dense innervation by DA-containing fibres.

Parahorizontal 300  $\mu$ m slices of the neostriatum from male albino rats (120–150 g) were maintained at the fluid interface of a perfusion chamber at 36 °C. Intracellular recordings were made with micropipettes filled with 2 M-potassium acetate (60–90 M $\Omega$ ). The mean resting membrane potential was  $-84 \pm 6$  mV (mean  $\pm$  S.D.,  $n = 62$ ) and all produced action potentials which overshoot 0 mV. The cells were silent at resting membrane potentials and only fired in response to depolarizing pulses (0.5–1 nA/80 ms) delivered through the intracellular electrode. Spike-frequency accommodation was observed when 500 ms pulses were applied. In cells depolarized from a holding potential of  $-65$  to  $-70$  mV, the resulting train of action potentials was followed by an afterhyperpolarization (AHP) which ranged from 1 to 5 mV in amplitude and 200–1000 ms in duration. The AHP was abolished after bath application of calcium-free medium or tetrodotoxin (1  $\mu$ M).

Ionophoresis of DA (0.5 M; 25–50 nA/3–5 min) close to the recording pipette resulted in two distinct actions. Firstly, as already described (Calabresi *et al.* 1987), it reduced the number of action potentials following intracellular stimulation, by increasing the threshold for spike initiation. Secondly, DA also inhibited the AHP; this affect was accompanied by an increase in the number of spikes per pulse. Whether these two actions of DA are mediated by a single receptor/membrane action is unclear. Nevertheless, they may help to reconcile the contradictory literature regarding the action of DA upon extracellular responses in striatal cells.

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AN AFTERHYPERPOLARIZATION IN NEOSTRIATAL CELLS RECORDED IN VITRO: EFFECT OF DOPAMINE. M. Garcia-Munoz, A. Rutherford, and G.W. Arbuthnott, MRC Brain Metabolism Unit, University of Edinburgh, United Kingdom.

A slow afterhyperpolarization (AHP) following a train of action potentials has been described in mammalian central neurons. Is there an AHP in striatal cells? If so, what is the effect of dopamine (DA) on the AHP? Parahorizontal 300  $\mu$ M slices of rat neostriatum were maintained at the fluid interface of a perfusion chamber at 36°C. Cells were recorded intracellularly (potassium acetate, 60-90 Mohms). No spontaneous activity was observed. The mean resting membrane potential was  $-83.7 \pm 6.4$  mV (n=82), depolarizing pulses (0.5-1 nA/80 ms) produced action potentials which overshoot 0 mV. After a train of action potentials an AHP followed in cells depolarized from a holding potential of -65 to -70 mV. The input membrane resistance did not change during the course of the AHP. The estimated reversal potential at 4.5 mM external potassium was  $-90 \pm 4$  mV. Iontophoresis of DA (0.5 M; 25-50 nA/3-5 min) resulted in several distinct actions: 1) As already reported (Calabresi et al., 1987), it reduced the membrane resistance with depolarization increasing the quantity of injected current necessary to reach the voltage of the firing level. 2) Induced accommodation to repeated cell firing. 3) Decreased the size of the AHP with an associated increase in the number of spikes. These results may help to reconcile the excitatory versus inhibitory effects of DA previously reported in the literature.

## 34.13

LAMINAR ORIGIN OF PATCH- AND MATRIX-DIRECTED CORTICO-STRIATAL PROJECTIONS IN THE RAT. C.R. Cerfen and A. Rutherford\*. Lab of Cell Biology, National Institute of Mental Health, Bethesda, MD and MRC Brain Metabolism Unit, Edinburgh, Scotland.

Cortico-striatal projections of medial frontal cortical areas in the rat are examined with the *Phaseolus vulgaris*-leucoagglutinin (PHA-L) anterograde axonal tracing method. Previous studies have suggested that cortico-striatal inputs to the striatal patch and matrix compartments are determined by the cortical area of origin; i.e. the prefrontal cortex projects to the striatal patches whereas the anterior cingulate and medial agranular cortices project to the striatal matrix (Cerfen, 1984; Donoghue and Herkenham, 1986). The present more detailed analysis suggests a variation on this scheme. PHA-L labeled cortico-striatal efferents of the infralimbic, prefrontal, anterior cingulate and medial agranular motor cortices each provide inputs to both the striatal patch and matrix compartments that are related to the laminar origin of the projection neurons. PHA-L injections into each of these cortical areas that label the efferents of cortical layer VI and deep layer V neurons, which provide a relatively restricted input to the deeper layers of the contralateral homotypic cortex, project relatively specifically to the striatal patch compartment. Conversely, injections that label efferents of more superficial cortical layers, which label a robust input to superficial layers of the contralateral homotypic cortex, label inputs to the striatal matrix. Also, qualitative observations suggest that inputs to the patch compartment are denser from the infralimbic and prefrontal cortices, whereas matrix directed projections are denser from anterior cingulate and medial agranular motor cortical areas. Thus the relative density of patch- and matrix-directed cortico-striatal neurons may vary among cortical areas.